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REPORT OF THE
MOSQUITO CONTROL COMMITTEE

January, 1983

Prepared by

RESEARCH DIVISION
State Department of Legislative Reference
Maryland General Assembly
Annapolis, Maryland 21401

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I. COMMITTEE CHARGE

The Mosquito Control Committee was created by the Governor pursuant to Joint Resolution 30 of the 1982 Session. Its charge was:

> "To study and investigate methods of controlling the spread of mosquitos in Somerset and Dorchester Counties."

II. MEMBERS OF THE COMMITTEE

After the passage of HJR 30, the charge of this Committee was given to the House Environmental Matters Committee. The Committee met on May 25, 1982 to receive testimony on the problem and methods of mosquito control. Present at that meeting were:

W. Henry Thomas, Chairman

Kay G. Bienen	Daniel J. Minnick, Jr.
Raymond A. Dypski	Catherine I. Riley
Marilyn Goldwater	Lewis R. Riley
Sheila E. Hixson	Steven V. Sklar
Paula C. Hollinger	Judith C. Toth
James E. McClellan	Michael H. Weir
	Gerald W. Winegrad

Robert H. Forste, Ph.D., Staff

A site visit, scheduled for August to the Blackwater Wildlife Refuge and other areas affected by mosquito populations and spraying programs, was eventually cancelled because it conflicted with the Special Session of the Legislature.

III. SYNOPSIS OF HEARING AND FINDINGS

Three major techniques for the control of mosquitoes were addressed: (1) innovative controls through the use of ultra-thin organic water surface films to "drown" both mosquito larvae and pupae and flood their breathing tubes; (2) the use of traditional pesticides (principally, the organophosphate Abate® or Temephos); and (3) open water marsh management.

The use of Arosurf® 66-E2 on Florida saltmarshes and in Thailand and Indonesia was described by Dr. Richard Levy (Research Entomologist, Lee County Mosquito Control District, Florida) and Mr. William Garrett (Head, Interface Chemistry Division, Naval Research Laboratory, Washington, D. C.). They presented a film, showing both laboratory and field tests of this non-toxic monomolecular film which is a larvicide and pupicide. The physical characteristics, effectiveness and cost aspects of this material were described (please see Appendix A for details); in addition to being non-toxic to fish and wildlife, relatively small quantities of this biodegradable film are required to obtain control (on a per-acre basis). The State of Florida has approved the use of this compound on waters of that State other than drinking water impoundments/supplies and shellfish propagation/harvesting areas. EPA registration and approval of this compound is expected in early 1983. Costs of application are approximately $6.50/acre (at applications of 0.5 gallons/acre).

Secretary Wayne A. Cawley, Jr. and Dr. Stanley R. Joseph of the Maryland Department of Agriculture described the program and practices of the Mosquito Control Section. Both spraying with organophosphate insecticides (principally Abate® and open water marsh management are utilized). Secretary Cawley indicated that two of their current concerns were (a) funding of their program (federal funds are unavailable for mosquito control) and (b) the reluctance of some property owners in affected areas to permit ditching. The Department of Natural Resources has contributed to the efforts of MDA in the past.

Dr. Joseph described integrated mosquito control in Maryland wetlands. In addition to reducing the annoyance to people, their objectives include the preventing of the transmission of encephalitis and dog heartworm. The Maryland Mosquito Control Advisory Committee reviews their program; it is comprised of representatives from MDA, the U. S. Fish and Wildlife Service; the EPA; U. S. Corps of Engineers; National Marine Fisheries Service; the Maryland DNR: and the University of Maryland. Their program includes training sessions for personnel, surveillance/monitoring of larvae and adult mosquitoes, and three major control efforts:

(1) Chemical control - ground or aerial applications of insecticides, as temporary control activities. Applications of insecticides such as Abate® cost about $1.60/acre; about 50,000 acres have been sprayed per year.

(2) Applications of BTI (a biological agent that kills mosquitoes), and expected field testing of monomolecular films (such as Arosurf® 66-E2).

(3) Open water marsh management (source reduction) - grid ditching (and other components of tidal, semi-tidal, and non-tidal) management techniques are being evaluated. Open water marsh management is the most important phase of MDA's control program. Over 6900 acres of mosquito breeding salt marsh in four counties were ditched from 1977 to 1980 at an average cost of $126/acre.

Dr. Joseph's complete report on integrated mosquito control, including cost data, is provided in Appendix C.

Testimony was received from Messrs. Wingate (Dorchester County) and Butler (Somerset County), regarding their concerns of solving the mosquito problem in their respective counties; financial aid to this end; and cooperation between federal, state and county agencies in mosquito control. Mr. Elvin Thomas, of the Dorchester County Highway Department, testified to the effectiveness of the use of Abate®.

Several muskrat trappers were present, and expressed their concern regarding the decline in muskrat populations, believing that the use of Abate® in mosquito control has contributed to declining muskrat harvests. Mr. Robbins indicated that muskrat harvests in his purview had declined as follows:

1977	4,000	muskrats
1979	1,800	muskrats
1980	121	muskrats
1982	16	muskrats

Correspondence and scientific documentation regarding the toxicity of Abate® to non-target species subsequent to the hearing leaves the issue unresolved. U. S. Department of the Interior (Fish and Wildlife Service, Patuxent Wildlife Center) studies indicate that Abate® is not a causative agent in mortality or reproduction capacity of muskrat(and/or other wildlife and fish) populations.

Conversely, some scientific articles indicate that Abate® causes signs, symptoms and the type of death in laboratory animals at rates higher than those used in marsh applications, typical of symptoms associated with other organophorous compounds. Articles and correspondence regarding this issue are provided in Appendix B.

The recommendations of the Committee were that present funding levels for mosquito control be maintained; that additional funds for mosquito control be sought; and that a portion of available funds be devoted to further field trials of monomolecular films (specifically, Arosurf® 66-E2).

TECHNOLOGY TRANSFER
FACT SHEET NAVY

APPENDIX A

BACKUP DOCUMENTATION PACKAGE

for

Fact Sheet Article

"Film Use Drowns Mosquitos"

Vol. 7, No. 1, Item 2
January 1982

For further information, please contact

R. Fulper, Code 1432
Naval Research Laboratory
Washington, DC 20375
Phone: (202)767-3744
Autovon: 297-3744

NRL
Science for the Navy and the Nation

THE
NAVAL RESEARCH LABORATORY
INFORMATION SERVICES BRANCH
CODE 2610
WASHINGTON, DC 20375

Release # 5-2-81C
Contact: Lloyd F. Carter

Telephone: (202) 767-2541
For Release: 16 Apr. '81

To be Tested in Southeast Asia

UN TO EVALUATE NRL'S MOSQUITO CONTROL TECHNOLOGY

Washington, D.C. --- A Naval Research Laboratory environmentally safe mosquito control technique, which has proven effective against mosquito larvae and pupae in natural breeding sites in southwest Florida, will be evaluated by a United Nations technical agency in southeast Asia in the near future.

NRL's William D. Garrett, principal investigator in the research, says the agency has confirmed it will initiate several projects in Thailand and Indonesia to evaluate the method for the regional control of critical diseases transmitted by mosquitos in those areas. The first project will be conducted near Bangkok, Thailand.

Urban and rural field demonstration projects will be conducted by scientists of these tropical countries to examine Garrett's surface-film control of mosquitos known to transmit Japanese B encephalitis, malaria, and dengue hemorragic fever. If these tests are successful, longer-term projects for operational mosquito control will be initiated.

Garrett reports he is personally providing consultation to the UN during the planning and development phases of the projects being initiated in the developing countries of southeast Asia.

Garrett's mosquito control method uses ultra-thin organic surface films with long lifetime on the water surface to sink both larvae and pupae and flood their breathing tubes. The film-forming agents, which are non-toxic and do not contain petroleum, spread spontaneously over the surface of the body of water where

MORE

mosquitos are breeding. Adults emerging from pupae or landing on the water to lay eggs are wetted amd drowned when contacting the film on the water's surface.

Mortality rates of up to 99 percent were recorded in the recent Florida mosquito control tests conducted by Lee County Mosquito Control District entomologists, working in conjunction with NRL.

..30..

(Note: Official U.S. Navy Film, release by the Department of Defense, Available from the Information Services Branch, Code 2610, Naval Research Laboratory, 20375, upon request. '54' 16mm Color Master. Time 1:30)

William D. Garrett
P.I. - NRL.
202-767-3683

SHEREX

SHEREX CHEMICAL COMPANY, INC.

SUBSIDIARY OF SCHERING AG, WEST GERMANY

5777 FRANTZ ROAD • P.O. BOX 646
DUBLIN, OHIO 43017
TEL (614) 764-6500

May 18, 1982

Dr. Robert Forste
Deputy Chief Research Division
Maryland General Assembly
90 State Circle
Annapolis, MD 21401

Dear Dr. Forste:

In response to your request for information on Arosurf 66-E2, I am enclosing a data sheet, price list, and an overview on the acute toxicity testing. The toxicity review was prepared for the UN, State of Florida, and other responsible parties interested in evaluating our product.

Arosurf 66-E2 is commercially available. Most of the efficacy data and field observations have been made by Dr. Levy. He is sending you reprints of his publications.

Thank you for your interest. We are anxious to assist in Maryland's effort to establish safe larvacides. Please let me know when we may be of assistance.

Sincerely,

Arthur J. Wolf
Commercial Development

AJW/dj

Enc.

SHEREX

TECHNICAL DATA

®AROSURF 66-E2

SHEREX CHEMICAL COMPANY, INC.

POST OFFICE BOX 646 • DUBLIN, OHIO 43017
TEL (614) 764-6500

®AROSURF 66-E2
CAS #52292-17-8

OTHER NAMES	C.T.F.A., Isosteareth-2	
	Polyoxyethylene (2) Isostearyl Ether	
	ISA-20E	
DESCRIPTION	Two Mole Ethoxylate of Isostearyl Alcohol	
SPECIFICATIONS	Color Hellige	1 Max.
	pH, 1% in 50/50 Isopropanol/Water	6.0 - 8.0
	Moisture	1% Max.
	Hydroxyl Value	142-152
	Acid Value	1 Max.
	Appearance @ 25°C	Clear Liquid
	Melting Point (CTMP)	-3 to -7°C
TYPICAL PROPERTIES	Approximate HLB#	4.7
	Specific Gravity	0.911 (25°C/25°C)
	Weight Per Gallon	7.595 lbs. (25°C)
		910 g/1

AVAILABILITY AND PACKAGING

Shipping Point, Janesville, Wisconsin, U.S.A.

Bulk Quantities
4,000 - 5,000 gal. tankwagons
8,000, 10,000, 20,000 gal. tankcars
Bulk export containers provided as available.

Drums
55 gal. tight-head steel drums, DOT 17-E (or 17-H or export) with bungs of 2 in. and 3/4 in. Net Weight 410 lbs. (186 kg.), or 54 gals. (204.3 1. Export containers of 20 ft. 38 drums or 40 ft. 78 drums are available.

TOXICITY

The results of F.H.S.A. acute toxicity testing indicates that ®AROSURF 66-E2 is non toxic and non irritating to the skin and eyes.

11/80

May 14, 1982

CURRENT PRICE LIST

Arosurf 66-E2

Prices f.o.b. Janesville, Wisconsin

Prices	Carload/ Truckload	25 - TL	5 - 25	2 - 4	1 Drum
Per lb.	$ 1.55	$ 1.59	$ 1.63	$ 1.66	$ 1.72
For reference only equivalent pricing:					
Per gal.	11.77	12.07	12.28	12.60	13.06
Per drum	635.40	651.90	668.30	680.60	704.20

5 gallon price - $75 (38#). Drums contain 410# net (55 gals.)

Prices are subject to confirmation at the time of order placement. This special price applies to Arosurf 66-E2 to be evaluated as a larvacide by Mosquito Control Agencies.

EXECUTIVE SUMMARY

ENVIRONMENTAL AND PHYSIOLOGICAL ASPECTS OF AROSURF 66-E2 (ISA-20E)

BY

ARTHUR J. WOLF
COMMERCIAL DEVELOPMENT

APRIL 12, 1982

SHEREX CHEMICAL COMPANY, INC.
P. O. BOX 646
DUBLIN, OHIO 43017

TOXICITY STUDIES

ON

ALCOHOL ETHOXYLATES AND AROSURF 66-E2 (ISA-20E)

INTRODUCTION

At the request of interested parties involved in mosquito control in the State of Florida, UN, WHO, etc., Sherex has prepared a toxicity review on alcohol ethoxylate, which includes specific acute testing on Arosurf 66-E2 (ISA-20E). The data shows that Arosurf 66-E2 exhibits a low order of toxicity and falls at the low end of the toxicity spectrum in the broad class of alcohol ethoxylates. (AE). Arosurf 66-E2's insoluble nature in water (< 2.5 mg/l (ppm) does not appear to manifest a significant threat to marine life from a practice viewpoint.)

We understand this review is necessary because the interested parties are evaluating the use of Arosurf 66-E2 as a larvacide. Dr. Levy's, et al, publications point to the efficacy of the product at very low use levels, < 0.5 gal/acre. At that level of exposure, we calculate the concentration in the environment would be < 1.5 ppm (considering water at one foot depth/acre). Because of Arosurf 66-E2's insolubility, the 1.5 ppm is actually a monomolecular film of .47 ml/m^2 or .46 mg/m^2 on the water's surface. These calculations are at the maximum recommended use level of .5 gal/acre.

Please keep in mind the alcohol ethoxylate (AE) toxicity data reviewed includes all AE's, both water soluble and insoluble product types. The toxicity of soluble AE's to aquatic life is considerably higher than insoluble AE's. Arosurf 66-E2 tests on Bluegills and Daphnia demonstrated a similar behavior. Test results of the product applied to the water surface showed a low order of toxicity to the more sensitive Bluegill and Daphnia - well below maximum suggested use levels. Artificially dispersed in water, aquatic tolerance decreased.

The data reviewed on alcohol ethoxylates and specific acute toxicity tests on Arosurf 66-E2 implies that, because of the relatively low level of toxicity, low water solubility, low use levels with a surface application, and high rate of biodegradation, no apparent threat to the environment is anticipated. This conclusion is fully supported by the non-target field observations of Levy, et al.

BACKGROUND ON ALCOHOL ETHOXYLATES

Ethoxylated fatty alcohols have been widely utilized for over 20 years in cosmetic, detergent, and agricultural formulations. Estimated U.S.A. domestic consumption in 1981 of these compounds in such applications is 600-700 million lbs./year.

The wide-spread usage of ethoxylated alcohol nonionic surfactants in consumer products creates an obvious potential for human and environmental exposure. However, the actual exposure is low enough that the relatively weak biological activity of this class of surfactants has not been identified with any adverse human health or environmental effects. The low exposure is a result of the non-volatile characteristics of this class of compounds, low use concentrations, and biodegradability characteristics.

The human health and environmental effects of major surfactants which includes ethoxylated alcohols (AE) have been extensively documented and reviewed by A. D. Little under contract to the Soap and Detergent Association. The available information has been assembled and critically reviewed by A. D. Little in three reports:

1) Arthur D. Little, Inc., "Human Safety and Environmental Aspects of Major Surfactants", a report to The Soap and Detergent Association, May 31, 1977.

2) M. M. Goyer, J. H. Perwak, A. Sivak, and P. S. Thayer, "Human Safety and Environmental Aspects of Major Surfactants (Supplement), Feb. 20, 1981.

3) A. Sivak, M. Goyer, J. Perwak and P. S. Thayer, "Environmental and Human Health Aspects of Commercially Important Surfactants", Proceedings of the Tenth Northeast Regional American Chemical Society Meeting, Potsdam, N.Y., June 30 - July 3, 1980. In press.

Copies of these reports are included as reference.

Summary of A. D. Little Findings

"AE exhibits a low order of acute toxicity in laboratory animals. Oral LD_{50} values range from 870 to >25,000 mg/kg. . . . . Two separate chronic studies with rats indicated no significant treatment-related effects resulted from ingestion of up to 1% AE in the diet for two years. Tumor incidence in treated animals were comparable to controls. There is no evidence of mutagenic or teratogenic effects resulting from AE exposure. Use of certain AE as analgesics and anesthetics in human therapy have produced no untoward reactions. These data suggest that use of AE as a component of detergent formulations poses no threat to environmental quality or to human safety."

ACUTE TOXICITY TEST RESULTS ON AROSURF 66-E2

The test results to date on Arosurf 66-E2 support the conclusion reached by A. D. Little on alcohol ethoxylates. The initial result of acute tests done in 1968 states:

> "Under the conditions specified, the product has an estimated oral LD 50 of 20 grams per kilogram of body weight, a primary skin irritation index of 2.92 and eye irritation scores of 5.67, 3.00 and 2.67 at 24, 48 and 72 hours respectively.
>
> Within the meaning of the F.H.S.L.A., the product is non-toxic orally, non-irritating to the eye and non-irritating to the skin."

The results of acute tests completed in the past year using recent EPA protocol are outlined below: (Copies are enclosed.)

Acute Oral LD_{50} >5 g/kg (presently testing @ 20 g/kg) 5/82 Results, >20g/kg

Acute Dermal LD_{50} > 2 g/kg

Primary Skin Irritation 4.9, Moderately irritating @ 72 hours.

Primary Eye Irritation

Primary Eye Irritation Scores

	Unwashed	Washed
24 hrs.	3.2	3.0
48 hrs.	2.0	3.7
72 hrs.	1.5	4.0
96 hrs.	0.7	1.3
7 days	0.0	0.0

No corneal opacity: irritation reversible within 7 days.

Ames test Negative

Inhalation Negative at > 2.7 mg/l, maximum product Test Level Achievable (preliminary Verbal Report)

Tests on aquatic organisms have also been performed.

To interpret the acute test results correctly requires a comparison of test protocols. The EPA test protocol requires the product be solubilized or dispersed in water. Although Arosurf 66-E2 is essentially insoluble in water (< 2.5 mg/l), the tests were run on the substance as dispersible. The results obtained are:

96 hr. LC_{50} Sheepshead Minnow	>300 mg/l		Test substance solubilized in acetone and dispersed in seawater.
96 hr. LC_{50} Mysid Shrimp	9 mg/l		
96 hr. LC_{50} Rainbow Trout	46 mg/l		
96 hr. LC_{50} Bluegill	85 mg/l		Test substance dispersed in water
48 hr. LC_{50} Daphnia	0.67 mg/l		

Since Arosurf 66-E2 is essentially water insoluble, the primary mode of marine life exposure is at the water surface. To correctly simulate this exposure, Arosurf 66-E2 was applied to the water surface and the LC_{50} test on daphnia (a surface life) and bluegill were repeated. The results obtained are:

96 hr. LC_{50} Bluegill	>500 mg/l	Test substance added to water surface
48 hr. LC_{50} Daphnia	290 mg/l	

A dramatic decrease in toxicity is obviously demonstrated. Acute toxicity to bluegill is negligible, and for daphnia, tolerance increased several hundred times (432 times to be exact).

EPA EXEMPTIONS OF ALCOHOL ETHOXYLATES, WHICH INCLUDES AROSURF 66-E2

Alcohol ethoxylates are exempt from tolerance requirements under 40 CFR Section 180.1001 when used as surfactants in formulations applied to crops, etc. Insight as to the EPA's opinion of the toxicity of alcohol ethoxylates as a class may be seen in their comments on Shell's request for a minor expansion in Section 180.1001. From the EPA's cover letter:

> "It was the opinion of our toxicologists that the similarity was sufficient to justify a finding that no hazard would result by its use. Data were not submitted in support of the request by Shell Chemical Company nor were data considered necessary since the use of the chemical is generally recognized as safe for the purpose intended . . . "

The complete correspondence is included.

FDA CONSIDERATION

Although Arosurf 66 E-2 is not directly approved for any food use, similar alcohol ethoxylates are approved for a variety of uses. A listing is attached.

BIODEGRADATION OF ALCOHOL ETHOXYLATES AND AROSURF 66-E2

The A. D. Little study sums up the biodegradation of alcohol ethyoxylate (AE) as follows:

> "As a class, AE undergo extensive, relatively rapid primary and ultimate degradation both in the laboratory and under field conditions. Linear primary AE also show rapid ultimate biodegradation to CO_2 and H_2O. Secondary and slightly branched primary alcohol ethoxylates appear to be degraded somewhat more slowly than linear primary AI."

Sherex's tests confirm the A. D. Little synopsis. In lab tests, Arosurf 66-E2 was found to undergo biodegradation with bacteria obtained from a pond at Lee County, Florida in three days. This is also consistent with the Shell study, especially examples on Page 15, Table V. Sherex is testing two additional bacterias.

ADOL 85, THE INDICATOR OIL

A monograph on stearyl alcohol is attached for reference. Stearyl alcohol is the saturated version of oleyl alcohol, Adol 85, which is referred to as the "indicator oil" mentioned in Dr. Levy's papers to test the presence of the Arosurf 66-E2. The toxicity of the products are essentially the same.

NOTE: Where necessary for regulatory personnel, copies of all test and reference information is available.

A.J.W.

LEE COUNTY MOSQUITO CONTROL DISTRICT

813-694-2174
SUNCOM 525-1011
POST OFFICE BOX 06005
FORT MYERS, FLORIDA 33906

T. W. MILLER, JR., DIRECTOR

May 28, 1982

Dr. Robert Forste
Deputy Chief Research Division
Maryland General Assembly
90 State Circle
Annapolis, Maryland 21401

Dear Dr. Forste:

It was a pleasure meeting with you and the delegates of the Maryland General Assembly concerning the use of a non-toxic larvicide and pupicide for the control of mosquitoes (®Arosurf 66-E2) as a potential alternative to ®Abate in marshes.

As requested, I am enclosing reprints and publications in press concerning the efficacy, application techniques and methodology, and safety of the monomolecular film ®Arosurf 66-E2.

Please do not hesitate to contact me if I can be of further assistance.

Sincerely,

Richard Levy, Ph.D.
Research Entomologist

RL/ld

Enclosures

Lee County Mosquito Control District
Procedures for Field Testing ISA-20E
Against Immature Mosquitoes

Monomolecular surface film(s) evaluated:* ISA-20E mosquito control formulations. These materials are non-ionic, biodegradable, surface active chemicals that spontaneously and rapidly spread over a water surface. ISA-20E has been classified as non-toxic orally and non-irritating to the eyes and skin.

Susceptible species evaluated: *Aedes taeniorhynchus, Aedes infirmatus, Aedes aegypti, Culex nigripalpus, Culex quinquefasciatus, Psorophora columbiae, Psorophora ciliata, Anopheles crucians, Anopheles quadrimaculatus, Uranotaenia sapphirina and Uranotaenia lowii.*

Susceptible stages of development: Larvae (1st - 4th instar), pupae, and emerging adults. Pupae are significantly more sensitive than larvae.

Mode of action: ISA-20E-induced mortality of larvae and pupae is presumed to be due to physical factors, i.e. habitat surface tension reduction with subsequent wetting of tracheal structures and anoxia and not by classical chemical toxicity induced by many conventional larvicides. Therefore, resistance to this material is not expected to develop.

Type habitats evaluated: Salt-marshes/ma groves, roadside ditches, grassy swales, grassy fields, storm water retention/detention basins, drainage ditches, tires, water holding containers, sewage treatment systems, tidal ditches, and potholes.

Type water evaluated: fresh (rain), semi-brackish, brackish, sea water, sewage and industrial effluent.

Temperature range evaluated: 59 - 95° F.

Dosages: Based on calculated water surface area of mosquito habitat (expressed in milliliters (ml) ISA-20E per square meter (m^2) of habitat). Recommended surface dosages of 0.20 - 0.45 ml ISA-20E/m² or 0.21 - 0.48 gallons/acre. Persistence in habitat has been generally found to be directly related to dosage.

<u>Application equipment evaluated</u>: Conventional hand pump and hand held and truck mounted compressed air sprayers; Simplex aerial spray systems.

<u>Pre-treatment procedure</u>: Mosquito samples should be obtained approximately 1 hr before spray application in a conventional manner (pint dipper) and the number of larvae according to instar and pupae recorded. The number of egg rafts should also be noted. Data concerning wind speed and direction, rainfall, water temperature, water surface characteristics (e.g. the amount of emergent and floating aquatic vegetation and debris) and non-target observations should also be recorded when possible.

<u>Application procedures</u>: ISA-20E should be sprayed around the vegetative perimeter of a mosquito habitat as well as in several locations throughout habitat. This technique will assure penetration through heavily vegetative areas, therefore providing more uniform surface coverage. In general, application techniques will be dependent on habitat characteristics, e.g. amount of floating and emergent aquatic vegetation and organic scum and debris.

<u>Post-treatment procedures</u>: Mosquito sampling and data retrieval should be conducted in a manner consistent with pre-treatment procedures. Percentage mortality of larvae according to instar and pupae should be recorded at 24 hr intervals until the presence of ISA-20E is not detected on water surface due to natural degradation. It should be noted that data has indicated that ISA-20E-induced mortality of *Culex, Aedes, Anopheles, Psorophora,* and *Uranotaenia* spp. in excess of 90% can be acute (within 24 hr) or delayed (within 48 - 96 hr). Time-related mortality was shown to be related to water temperature, wind action, species and stage of development, and/or to the dissolved oxygen concentration of the mosquito habitat.

<u>Monitoring persistence of monomolecular surface films</u>: The presence of

ISA-20E mosquito control film can be detected in a mosquito habitat by using the appropriate ISA-20E indicator oil. At each post-treatment sampling period a drop of indicator oil should be applied in several locations around the vegetative perimeter of a habitat with a pipette to indicate the completeness of coverage and the stability of the mosquito control film in an area. The indicator oil will tightly bead on the water surface if the monomolecular film pressure is adequate for effective mosquito control. If the material has naturally degraded or has been translocated by wind action the indicator oil will visibly spread on the water surface. This will indicate that film pressure in this area is not satisfactory for effective control of immature mosquitoes. It should be noted that moderate unidirectional winds can displace or translocate the surface film to the downwind portion of a mosquito habitat. This condition, although usually temporary, will produce an area of highly compacted film and a larger area where essentially no film is present. Large numbers of larvae and pupae are usally physically translocated in this process. Wind-induced disruption of film integrity over the water surface in densely vegetative areas was significantly less than observed in open areas. It is important to note that ISA-20E will usually redistribute and reseal itself over all or most of the mosquito habitat when the wind speed decreases. Therefore, careful monitoring of the habitat with the indicator oil in upwind and downwind locations is essential. At the dosages recommended (i.e. 0.20 - 0.45 ml/m²) mosquito control film can be expected to be stable for 2 - 6 days after treatment depending on the surface dosage, type habitat, and evironmental conditions.

Retreatment of certain areas may be necessary when the indicator oil spreads under no or low (2 mph) wind conditions, thereby indicating that ISA-20E has failed to respread over mosquito habitat. In addition, it is recommended that habitats be retreated if 90% or greater mortality of immature mosquitoes is not obtained within 72 hr post-treatment. It should be noted that high concentration of dead (floating and/or submerged) larvae and/or pupae are usually obtained with dipper samples when high mortality is indicated. Furthermore, difficulty in surface coverage can be expected when applications are conducted in winds of 10 mph or greater or when undirectional winds persist for several days.

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*ISA-20E can be suspended in water with agitation at recommended dosages (0.21 - 0.48 gal/acre) and therefore be applied at a total rate of 5 gal/acre or greater to facilitate penetration in heavily vegetative areas.

ISA-20E (®Arosurf 66-E2) and indicator oil (®Adol 85) are manufactured for use as cosmetic ingredients by Sherex Chemical Co., Inc., Post Office Box 646, Dublin, Ohio 43017. - Art. J. Wolf, Manager, Commercial Development (phone 614-764-6614).

Questions concerning these procedures should be directed to:

Dr. Richard Levy
Research Entomologist
Division of Biological Control
Lee County Mosquito Control District
Post Office Box 06005
Fort Myers, Florida 33906

Control of Immature Mosquitoes Through Applied Surface Chemistry

R. LEVY[1], J. J. CHIZZONITE[1], W. D. GARRETT[2] and T. W. MILLER, JR.[1]

[1]Lee County Mosquito Control District, P. O. Box 06005,
Fort Myers, Florida 33906
[2]Environmental Science Division, Naval Research Laboratory
Washington, D. C. 20375

ABSTRACT

The utilization of monomolecular organic surface films for mosquito control is discussed with special reference toward the mode of action, efficacy, and safety of isostearyl alcohol containing two oxyethylene groups.

One technique for the control of mosquitoes that has been used successfully throughout the years has been to prevent the emergence of the adults from its aquatic breeding habitat through the use of petroleum-based oils. These oils presumably suffocate larvae and pupae by preventing normal access to oxygen at the air-water interface and/or by specific toxicity. Oil soluble, surface-active agents have also been formulated with certain petroleum oils to promote uniform spreading over the surface of the water and to enhance respreading after disruption by wind. For the most part, application rates of diesel-based larviciding oils have been high i.e. 5 gal/acre or greater to achieve effective control of immatures under field conditions. However, the escalating cost of petroleum oil as well as the phytotoxicity and the detrimental effects on certain non-target aquatic organisms at high application rates have led to a significantly curtailed use of petroleum-based larviciding oils.

One potential alternative to the aforementioned approach has been to control immature mosquitoes in the aquatic environment by the physiocochemical modification of the water interface with low dosages of non-petroleum monomolecular organic surface films. When applied to a mosquito habitat, some monomolecular organic surface films can modify the physical properties of water surfaces in ways which interfere with the normal activities and development of mosquito eggs, larvae, pupae, and/or emerging adults. These films can significantly reduce the surface tension of a mosquito habitat and subsequently kill larvae and pupae by inhibiting proper orientation at the air-water interface and/or by increasing the wetting of tracheal structures and causing anoxia. The sinking and inactivation of egg rafts laid at the water surface by monomolecular organic films have also been demonstrated.

For the most part, research on the utilization of various soluble and insoluble monolayers for the control of immature mosquitoes has been conducted in the laboratory. However, an effective and standard bioassay procedure, for the evaluation of monolayers was not used in these tests. Therefore, results were misleading and confusion was encountered when trying to extrapolate performance to the field. Although the results of several laboratory studies have shown that certain monomolecular organic surface films may have practical application for operational mosquito control, significant data have not been generated to indicate the field effectiveness of most monomolecular surface films against a wide variety of mosquito species and stages of development under natural breeding conditions.

During the past 2 years we have modified former bioassay procedures and have evaluated over 30 types of monomolecular organic surface films in the laboratory to determine their potential as mosquito control agents (Levy et al. unpublished). Although 15 of these surface films have met the criteria established by Garrett and White (1977) for selection of an effective monolayer based on physical and chemical parameters and have shown good activity when bioassayed against the immature stages of *Anopheles* mosquitoes, only five have shown efficacy when evaluated against the larvae and pupae of several species of mosquitoes under natural field conditions. Four of these five monomolecular organic surface films are in preliminary to intermediate stages of field testing; however, the monomolecular organic surface film isostearyl alcohol containing two oxyethylene groups (ISA-20E)[3] has been extensively evaluated at the Lee County Mosquito Control District in natural mosquito habitats.

ISA-20E is a biodegradable, non-petroleum vegetable-based oil used in the cosmetic industry. It is essentially colorless and odorless, resists oxidative rancidity (i.e. should have a long shelf-life), has a melting point of −5 C (i.e. can be used in the field under a wide range of temperature extremes), is non-ionic (i.e. resists solubility losses into saline water due to the formation of soluble organic salts), and has the property of autophobicity, (i.e. the material will not spread over its own monomolecular film). The excess liquid remains as a thick, oil-like lens in equilibrium with the spread film. When portions of the film have been degraded or displaced by natural forces, the excess material in the lens or reservoir immediately and spontaneously spreads to restore the equilibrium between the film and the excess bulk liquid, thereby maintaining uniform coverage and film pressure.

ISA-20E spreads into a uniform, nearly monomolecular layer over the water, and thus cannot be seen because it is too thin to absorb light or cause irridescence due to reflective interference. However, it is necessary to determine the presence of the film and insure that it exists at a sufficiently high film pressure (i.e. surface tension reduction of less than 29 dynes/cm) to be physically effective against larvae, pupae and emerging adults. This determination can be made by adding a drop of a refined grade of oleyl alcohol[4] to the water surface in several locations throughout a mosquito habitat to act as a spreading oil to indicate the completeness of coverage by the mosquito-control film (i.e. ISA-20E). If the drop of oleyl alcohol does not spread (i.e. forms a bead on the surface of the water), the film is intact and highly compressed. Should spreading of the indicator oil occur, a silvery or slightly colored spreading pattern is observed. Although the surface pressure exerted by oleyl alcohol is about 10 dynes/cm less than that of the control film, it is still quite suitable for indicating purposes, because the surface pressure of a film on water changes rapidly as film loss occurs. Spreading of the oleyl alcohol drop will indicate control-film depletion within seconds of its occurrence. In fact, the indicator drop will spread even though the control film is present when the pressure of the control film has fallen below that of the spreading oil (surface tension reduction of 31 dynes/cm). For these reasons spreading of the indicator oil may be taken as meaning that the control film is either absent from the zone tested or is not sufficiently compressed to be effective for mosquito control. Unlike insect growth regulators, persistence of ISA-20E in the field can be monitored at various post-treatment intervals. This is an important operational tool for use in backchecking an area to determine if retreatment is necessary.

Field trials at the Lee County Mosquito Control District during 1978-1980 with ISA-20E have indicated that this surface active chemical is highly effective in controlling larvae, pupae, and/or emerging adults of *Anopheles quadrimaculatus, An. crucians, Aedes aegypti, Ae. taeniorhynchus, Ae. sollicitans, Ae. infirmatus, Psorophora columbiae, Ps. ciliata, Culex nigripalpus, Cx. quinquefasciatus, Uranotaenia lowii* and *Ur. sapphirina* in polluted, fresh, brackish and/or sea water habitats of Lee County, Florida. (Levy et al. 1980, 1981a, b). For the most part, effective surface dosages for larval control have ranged from 0.30-0.45 ml ISA-20E/m^2 (0.32-0.48 gal/acre). Tests have indicated that control of pupae can be achieved at one-half the recommended range of dosages for larval control. At these dosages 90-100% control of larvae and pupae of the aforementioned species usually resulted before biodegradation of the film was indicated.

Ground and aerial application of ISA-

[3]ISA-20E is manufactured for use as a cosmetic ingredient by Sherex Chemical Company, Inc., P. O. Box 646, Dublin, Ohio 43017, under the trade name of ®Arosurf 66-E2.

[4]Oleyl alcohol indicator oil is manufactured for use as a cosmetic and pharmaceutical ingredient by Sherex Chemical Company, Inc., P.O. Box 646, Dublin, Ohio 43017, under the trade name of ® Adol 85.

20E to control immature mosquitoes have been demonstrated at our district. Our field trials utilizing hand-held sprayers, truck mounted compressed air sprayers, as well as a Bell 47G helicopter equipped with a Simplex aerial spray system, have indicated that ISA-20E can be applied effectively by conventional application techniques. Field tests with ISA-20E at surface dosages of 0.27-0.48 gal/acre have indicated that this monolayer is not phytotoxic and that it will cause little or no adverse affects on fish and wildlife exposed to the material during mosquito control operations. Laboratory and field tests (Levy et al. 1981a) with ISA-20E have indicated that it is compatible with certain parasites, pathogens, and predators and therefore this monomolecular film may be employed in integrated mosquito control programs. Additional tests (Levy et al. unpublished) have indicated that this monolayer does not affect water temperature and the rate of evaporation as well as gas exchange at the air-water interface. ISA-20E has been classified as non-toxic orally and non-irritating to the eyes and skin and therefore, the use of this monomolecular organic surface film is not expected to create a health hazard to man or cause detrimental environmental effects.

The mode of action of ISA-20E on mosquito larvae, pupae, and emerging adults is presumed to be due to physical rather than toxic factors, i.e. habitat surface tension reduction with subsequent inhibition of surface orientation and/or wetting of larval or pupal tracheal structures and death by anoxia. Therefore, continued field expousre of mosquitoes to ISA-20E is not expected to cause the classical resistance observed with several conventional toxicants used for larviciding. Mortality induced by ISA-20E on immature mosquitoes in excess of 90% has been shown to be acute (i.e. within 24 hr) or delayed (i.e. within 48-72 hr) and was related to physiological and morphological difference in mosquitoes, stage of development, wind action, and/or the amount of dissolved oxygen in the mosquito habitat. Since mortality can be delayed, the use of the indicator oil plays an important role in the operational use of this material. In general, laboratory and field tests have indicated that pupae are significantly more sensitive to ISA-20E than larvae, however, all instars were shown to be susceptible. For the most part, results of over 60 field trials with ISA-20E have indicated that the only significant limiting factor for effective acute or delayed control of immature mosquitoes was persistent, unidirectional winds of high velocity. It should be noted that several solid and liquid formulations have been developed to compensate for this effect as well as to increase the rate of larvicidal action of ISA-20E. These materials are currently being evaluated in the field (Levy et al. unpublished).

Data from field trials have indicated that persistence (stability) of ISA-20E is directly related to dosage and possibly related to habitat water quality (i.e. the degree of microbial degradation) and water temperature. Observations have indicated that spray applications of ISA-20E can persist in certain field situations and kill mosquito larvae and pupae for a week or longer when applied at a surface dosage as low as 0.3 ml/m^2 (0.32 gal/acre). It should be noted that preliminary field tests with a solid formulation of ISA-20E have shown that a stable and effective mosquito-controlling film can be slow-released for several months under natural conditions. (Levy et al. unpublished).

In general, cost considerations as well as the results of research at the Lee County Mosquito Control District for the past 2 years have indicated that in many instances the monomolecular organic surface film isostearyl alcohol containing two oxyethylene groups can be an acceptable alternative to certain petroleum-based oils and insect growth regulators for the operational control of larvae and pupae in their natural habitats. Under certain situations, this monomolecular surface film may also be a suitable substitute for other larvicides.

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CONTROL OF IMMATURE MOSQUITOES WITH LIQUID AND SOLID FORMULATIONS OF A MONOMOLECULAR ORGANIC SURFACE FILM[1,2]

R. Levy[3], C. M. Powell[3], W. D. Garrett[4] and T. W. Miller, Jr.[3]

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[2]Mention of a brand name or proprietary product does not constitute a guarantee or warranty by Lee County Mosquito Control District, and does not imply its approval to the exclusion of other products that may also be suitable.

[3]Lee County Mosquito Control District, P. O. Box 06005, Fort Myers, Florida 33906.

[4]Environmental Sciences Division, U.S. Naval Research Laboratory, Washington, D.C. 20375.

Abstract

Research and development of liquid and solid formulations of the monomolecular organic surface film ®Arosurf 66-E2 for mosquito control are discussed, with emphasis on their potential implementation in operational mosquito control programs.

In 1977 the Lee County Mosquito Control District initiated an applied research and development program aimed at evaluating alternative techniques for currently used conventional practices. The main objective was to determine if certain biological control agents and non-toxic chemicals could be integrated into our operational program and thereby reduce the use of conventional toxicants as well as solve specific operational problems that were characteristic to our area.

Since the onset of our program we have conducted research in areas of parasite, predator, and pathogen field efficacy and application technology as well as in mass production and storage. Several of these biological control agents are mass-reared at our district and are expected to become an integral part of our operational program.

One on-going research project has involved the extensive evaluation of non-petroleum monomolecular organic surface films as potential larvicides and pupicides. The dramatic escalation in the cost of petroleum oils used for larviciding and pupiciding as well as the reported phytotoxicity and adverse effects on selected non-target organisms by diesel-based larviciding oils were the initiating factors for this research. Since the onset of the project we have evaluated over 50 types of organic surface films in the laboratory and field and have determined that one surface active chemical, i.e., isostearyl alcohol containing two oxyethylene groups (designated ISA-20E) is cost/effective and can be an acceptable alternative to the use of petroleum based oils as well as other larvicides used in mosquito control (Levy et al. 1980 a, b; 1981; 1982 a, b).

ISA-20E Liquid Formulations

ISA-20E is manufactured as a cosmetic ingredient by Sherex Chemical Company under the trade name of ®Arosurf 66-E2[5]. The efficacy of this product in mosquito

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[5]Sherex Chemical Company, Inc., P. O. Box 646, Dublin, Ohio 43017.

control as well as the effectiveness of the oleyl alcohol indicator oil[6] to monitor field persistence of ISA-20E have been demonstrated at several mosquito control districts in Florida as well as by mosquito control agencies in other areas of the United States and Overseas. ISA-20E is currently being evaluated by ground and helicopter application on an operational basis at certain mosquito control districts in Florida under a permit granted to the Office of Entomology, State of Florida, by the Department of Environmental Regulation for use of ISA-20E as a larvicide and pupicide. Material cost per acre for ISA-20E at typical application dosages of 0.2 - 0.4 gal/acre is estimated at $2.50-5.00 . Control of pupae can be accomplished at a rate of 0.1-0.2 gal ISA-20E/acre at an estimated per acre cost of $1.25-2.50. These costs are presently cheaper than typical mosquito control application rates of the Florida diesel-based larviciding formulation and FLIT MLO®, and are competitive with several other chemical and biological control agents used to control immature mosquitoes. It should be noted that higher dosages of ISA-20E, i.e., 0.75 - 1.0 gal/acre +, may also be cost/effective when used in dynamic permanent water *Culex* and *Anopheles* spp. habitats where highly prolonged film persistence (hence mosquito control) would be desirable. In addition, overdosing can be an acceptable procedure in certain situations where lateral expansion of semi-permanent mosquito habitats (eg. *Aedes* and *Psorophora* spp.) due to persistent rainfall is expected to occur within a few days after initial surface application. In this case, high mosquito-controlling film pressure can be maintained in the newly flooded breeding areas without reapplication of the material.

The larvicidal and pupicidal action of this surface film have been shown to be physical and not toxic in nature, being related to habitat surface tension re-

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[6]Manufactured as a cosmetic and pharmaceutical ingredient under the trade name of ®Adol 85 N.F. by Sherex Chemical Company, Inc., P. O. Box 646, Dublin, Ohio 43017.

duction and subsequent wetting of tracheal structures leading to suffocation. Therefore, resistance of mosquitoes to ISA-20E is not expected to develop.

Field trials at the Lee County Mosquito Control District have indicated that ISA-20E can be applied by conventional ground and aerial spray equipment as technical chemical at dosages ranging from ca. 0.20 - 0.50 gal/acre or at these recommended dosages as an agitated ISA-20E water-based suspension at final application rates of 5-7 gal/acre to effectively control larvae, pupae, and emerging adults of *Aedes, Anopheles, Culex, Psorophora,* and *Uranotaenia* spp. with little or no adverse environmental effects.

It should be noted that when ISA-20E is suspended in water with agitation, it is recommended that the system be thoroughly cleaned of petroleum oils (eg. diesel) and conventional pesticide residues prior to introduction of the ISA-20E. Diesel plus ISA-20E plus water can produce a milky gelatinous unsprayable material. ISA-20E can become contaminated with residues of pesticides left in the spray tank from prior use which may result in undesirable environmental and non-target stress. Spray systems can be cleaned with certain solvents (eg. 2-propanol) or with multiple washings of ISA-20E-water suspension. Detergents (soaps) are excellent for cleaning ISA-20E from hands, clothing, etc., but are not recommended for cleaning ISA-20E from spray tanks. Detergent residues remaining in the tank can act to destroy the monomolecular integrity of ISA-20E and will therefore affect normal field persistence and spreading/respreading characteristics of ISA-20E.

Vigorous agitation of ISA-20E in the water will assure a homogeneous suspension and accurate application rates. Conventional paddle agitation and agitation by pump recirculation have been shown to produce effective results in truck and helicopter spray systems, respectively. It should be noted that the addition of a 30 gal/min submersible pump in a Bell 206 helicopter spray tank was shown to significantly improve agitation over the use of the normal 10 gal/min recirculation pump by-pass rate.

Additional research by Hertlein et al. (unpublished) has also shown that recommended dosages of ISA-20E can be injected into a stream of water for application at high spray pressures and volumes with the use of commercially available injection valves. It is expected that these new application procedures will greatly increase the range of effectiveness of ®Arosurf 66-E2 in mosquito control programs.

ISA-20E entrapment of ovipositing females and resting males has been demonstrated in the laboratory and observed under field conditions. The sinking and inhibition of eclosion of egg rafts of *Culex* spp. have also been demonstrated in bioassays as well as under field conditions with the use of high pressure spray equipment. Therefore, under certain situations monomolecular films of ISA-20E can exert an ovicidal, larvicidal, pupicidal, and/or adulticidal effect on natural mosquito populations.

The larvicidal action induced by this surface film of 90% or greater was observed to occur within 24 hr post-treatment (i.e., acute kill), however, under most field conditions this level of mortality was usually obtained within 48-72 hr post-treatment (i.e., delayed kill). For the most part, the rate of mortality was attributed to species, instar, stage of development, habitat oxygen levels, habitat surface characteristics, and climatological factors. High pupal mortality usually occurred 2-4 hr after treatment. Prolonged persistence of ISA-20E at high film pressure under most environmental conditions was shown to produce the sustained physical impact on immatures when an acute larvicidal effect was not achieved. ISA-20E has been reported to persist in a variety of natural habitats from 2-10 days at recommended dosages. However, at any dosage, sustained wind fetch was observed to adversely affect application and uniform water surface coverage by translocating and compacting the ISA-20E in downwind areas. This was compounded when high concentrations of floating organic and inorganic debris and vegetation was present in the habitat. When wind velocity was high and directional for extended periods, respreading of ISA-20E to displaced areas could not occur and subsequent control of immatures in locations of little or no film pressure was poor. For the most part, this was not a significant problem under conditions of fluctuating wind speed and direction; however, a delayed larvicidal response was usually observed. Conditions such as drainage, overflow and runoff have also been shown to adversely affect film performance in certain habitats.

ISA-20E Solid Formulations

Since mortality of natural populations of larvae exposed to ISA-20E could be delayed and was therefore a function of film persistence, research was conducted to determine if a solid biodegradable matrix could be developed for the release of monomolecular films of ISA-20E for the maintenance of high film pressure on immature mosquitoes for prolonged periods and thereby compensate for the limiting effects of persistent wind, runoff, and overflow. To effect the desired dispersal mechanism, ISA-20E was formulated with several dispersants, binders, and stabilizing components. The resultant solid/semi-solid matrices were evaluated under laboratory and field conditions against larvae and pupae of *Anopheles, Culex, Aedes,* and *Psorophora* spp. to determine if a stable mosquito-controlling monomolecular film could be consistently released for extended periods. The following set of criteria were the basis of evaluating the performance of over 25 solid formulations. An effective solid formulation of ISA-20E should: (1) Be composed of non-ionic and non-reactive components for use in a variety of water quality situations; (2) release surface films for prolonged periods under a wide range of temperatures; (3) release surface films for prolonged periods after exposure to drying and re-flooding conditions; (4) maintain a solid consistency and release minimal dosages after long-term field exposure; (5) release monomolecular films upon contact with water with little or no delay; (6) have a persistent larvicidal and pupicidal effect against a wide variety of mosquito species; (7) be non-toxic, biodegradable, and produce little or no adverse environmental impact after prolonged field use; (8) be capable of being fabricated into beads, pellets, blocks and cylinders for a variety of application techniques; (9) have a stable shelf-life after prolonged storage at ambient temperatures; (10) and be composed of materials that are commercially available at a reasonable cost.

Initial field trials were conducted with 3 floating solid formulations containing 30-50% ISA-20E that were anchored in an upwind location in 70 m^2 standing paludal ponds containing various amounts of floating and emergent vegetation. Results of these tests against mixed natural populations of *An. quadrimaculatus*, *An. crucians*, and *Cx. erraticus* indicated that ISA-20E could be released from a 280-290 g biodegradable matrix for over 5 months under field conditions and control 90-100% of the immatures. It should be noted that weekly spray treatment of ISA-20E at a dosage of 0.4 gal/acre was required to achieve this level of control. Although prolonged film release with the solids were demonstrated, the matrices softened too rapidly and released an excess amount of surface film. Therefore, these formulations were not suitable for operational mosquito control. In cooperation with Sherex Chemical Co., we have greatly improved the consistency of the matrix and the rate of film release. To date, over 25 solids have been evaluated in the laboratory against *Culex* and *Aedes* spp. to determine which formulations adequately meet the criteria we have established for an effective solid.

Laboratory tests against larvae and pupae of *Ae. aegypti* with several 0.5 - 7 g solid formulations containing 40-50% ISA-20E have indicated that a stable and effective mosquito-controlling surface film can be maintained in containers for 2-5 months and produce cumulative mortality of larvae, pupae, and emerging adults of 90-100%. Similar results were obtained in tests against *Ae. taeniorhynchus* and *Cx. quinquefasciatus*. The entrapment of ovipositing females of *Cx. quinquefasciatus* on surface films of ISA-20E released by the solids has also been demonstrated.

Preliminary field tests in salt-marshes against *Ae. taeniorhynchus* with 5-15g cubes and 280-290g cylinders of these formulations indicated that monomolecular films of ISA-20E can be released for extended periods to control larvae and pupae in semi-permanent habitats. Results indicated that certain formulations are stable enough to release surface films after being subjected to periods of drying and reflooding. Similar results were obtained in tests against *Ps. columbiae* and *Ps. ciliata*. It should be noted that the effective use of the indicator oil in monitoring the release of ISA-20E from solid formulations and its persistence in a mosquito habitat have been demonstrated. Although the results of tests with these formulations are extremely promising and indicate their potential use in certain situations for season-long mosquito control from one application, problems concerning matrix dissociation after prolonged exposure to water have to be solved before reliable formulations can be developed for use in operational programs. Field trials with new solids are scheduled for this summer. It should be noted that ISA-20E intermittent drip-dispensing systems can be used to maintain persistent film pressure for several months in habitats such as paludal ponds and irrigation and sewage treatment systems.

In summary, research at the Lee County Mosquito Control District has shown that ®Arosurf 66-E2 is a safe and effective product for use in mosquito control programs. Solid formulations of this monomolecular surface film are expected to greatly increase the range of its effectiveness as well as reduce application costs.

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GROUND AND AERIAL APPLICATION OF A MONOMOLECULAR ORGANIC SURFACE FILM TO CONTROL SALT-MARSH MOSQUITOES IN NATURAL HABITATS OF SOUTHWESTERN FLORIDA[1]

R. LEVY[2], J. J. CHIZZONITE[2], W. D. GARRETT[3] AND T. W. MILLER, JR.[2]

ABSTRACT. The efficacy of monomolecular organic surface films of isostearyl alcohol containing 2 oxyethylene groups was evaluated at varying dosages against natural populations of *Aedes taeniorhynchus* Wiedemann in several salt-marsh habitats. Results of ground and aerial spray application at surface dosages of 0.30 – 0.45 ml/m² (0.32 – 0.48 gal/acre) indicated that this chemical can be used effectively to control larvae and pupae of this *Aedes* sp. under a wide range of field conditions. Methodology for field application, mode of action of this chemical on immature mosquitoes, and environmental factors which influence the surface-film method are discussed.

When applied to the surface of a mosquito habitat a thin film of isostearyl alcohol containing 2 oxyethylene groups (ISA-20E)[4] can disrupt the normal orientation of immature mosquitoes at the air-water interface by reducing the water surface tension to less than 29 dynes/cm. This physical process can kill larvae and pupae by increasing the wetting of tracheal structures and subsequently causing anoxia (suffocation). Also it can adversely affect the emergence of adult mosquitoes. Since ISA-20E-induced mortality of immature mosquitoes is presumed to be produced by physical factors and not by classical chemical toxicity induced by many conventional larvicides, resistance to this material is not expected to develop. This monomolecular organic surface film is a non-ionic, non-petroleum, biodegradable surface active chemical that should have a long shelf-life, can be used in the field over a wide temperature range, and will not spread over its own monomolecular film (Garrett and White 1977). Tests have indicated it has little or no adverse effects on mammals (Reynolds, personal communication) and several species of non-target aquatic organisms (White and Garrett 1977, Levy et al. 1980).

The efficacy of ISA-20E in controlling natural populations of larvae and pupae of *Culex nigripalpus* Theobald and *Cx. quinquefasciatus* Say in an industrial sewage treatment system was reported by Levy et al. (1980). Results of field trials in sewage settling, polishing, and evapo-percolation ponds indicated that 97% control of immature *Culex* spp. could be obtained at a spray dosage as low as 0.33 ml ISA-20E/m² water surface (0.35 gal/acre). Data from laboratory and/or simulated field studies have indicated that ISA-20E is highly effective in killing 4th instar larvae and pupae of *Anopheles quadrimaculatus* Say (White and Garrett 1977) and pupae of *Aedes aegypti* (L.) (Garrett 1976) at a surface dosage of 0.04 ml/m² (0.043 gal/acre). However, at the same surface dosage, they showed that ISA-20E was not effective in controlling larvae of *Ae. taeniorhynchus* Wiedemann (White and Garrett 1977) and *Ae. aegypti* (Garrett 1976).

[1] Mention of a brand name or proprietary product does not constitute a guarantee or warranty by Lee County Mosquito Control District, and does not imply its approval to the exclusion of other products that may also be suitable.

[2] Lee County Mosquito Control District, P. O. Box 06005, Fort Myers, Florida 33906.

[3] Environmental Sciences Division, Naval Research Laboratory, Washington, D.C. 20375.

[4] ISA-20E is manufactured for use as a cosmetic ingredient by Sherex Chemical Co., Inc., P. O. Box 646, Dublin, Ohio 43017, under the trade name Arosurf 66-E2.

In addition to the control of *Culex* spp. in polluted and fresh water areas, the major portion of the Lee County Mosquito Control District program is aimed at the control of flood-water mosquitoes breeding in semi-permanent habitats created by intermittent tides and/or rain. Although *Psorophora columbiae* Dyar and Knab is a severe pest during the rainy season, our principal target species throughout the year is *Ae. taeniorhynchus* which breeds in the 66,000 acres of coastal salt-marsh of Lee County, Florida. Therefore, investigations were conducted to determine the efficacy of ISA-20E for controlling larvae and pupae of *Ae. taeniorhynchus* in natural habitats of Lee County, Florida.

METHODS AND MATERIALS

Salt-marshes mainly characterized by white, black and red mangrove, cenicella, and brazilian pepper, as well as potholes, ditches, ponds, and fields breeding immature stages of *Ae. taeniorhynchus* were sprayed with varying concentrations of ISA-20E. The water surface area of the test habitats ranged from 6.5–3582.7 m^2.

Surface applications were made with a small plastic hand-activated pump sprayer (Levy et al. 1980) at dosages of 0.30–0.45 ml/m^2 (0.32–0.48 gal/acre). Aerial applications were made from a Bell 47G helicopter equipped with a Simplex® spray system having one 52 gal tank filled with ca. 2 gal of ISA-20E and a boom (9.75m) fitted with 18 TeeJet® nozzles (No. 6135) with D2 orifices. This spray system was calibrated to dispense ca. 0.4 gal ISA-20E/acre or 0.37 ml ISA-20E/m^2 water surface at a pump pressure of 11–12 psi. Three helicopter tests were conducted at an air speed of ca. 74 kmph and at an altitude of ca. 9–15 m. To achieve the desired surface dosage (ca. 0.37 ml/m^2), 2–4 spray swaths were flown (1 swath = ca. 15.2–18.3 m) over the test areas. Wind speed at the time of application was 12.9–16.1 kmph with gusts up to 25.7 kmph in test 1, 8.0–11.3 kmph in test 2, and <3.2–4.8 kmph in test 3.

About 1 hr prior to treatment with ISA-20E, 3–25 mosquito samples were obtained with a pint dipper from several locations in each test site. The number of mosquito samples collected was based on the size of the habitat as well as on the concentration and spatial distribution of immatures at the time of sampling. The numbers of *Ae. taeniorhynchus* pupae and larvae according to instar were recorded as well as certain physical and environmental measurements, meteorological characteristics, and water quality characteristics associated with each test site. Samples were obtained at 24, 48, 72, 96, and/or 144 hr post-treatment in a manner consistent with pre-treatment procedures. Persistence (stability) of ISA-20E was monitored at each post-treatment sampling period with an indicator oil in a manner previously described by Levy et al. (1980).

Percentage reduction of larvae and/or pupae at each sampling interval was the main criterion used to evaluate the efficacy of ISA-20E against *Ae. taeniorhynchus*. Data were evaluated according to stage of development and dosage by analyses of variance. The effect of ISA-20E on non-target vertebrate and invertebrate aquatic organisms was also recorded.

A series of bioassays was conducted against laboratory-reared larvae and pupae of *Ae. taeniorhynchus*. These tests were conducted in 400 ml glass beakers containing ca. 250 ml of 50% sea water (sea water diluted with distilled water) and 10 immature *Ae. taeniorhynchus*/beaker (2–3 replications/test). The salinity, conductivity, dissolved oxygen, and pH of the sea water ranged from 15.5–16.1 ppt, 26,800–27,000 μmhos/cm, 4.3–6.9 ppm, and 6.5–7.6, respectively. ISA-20E was applied to each beaker with a microsyringe at a surface dosage of ca. 0.25 ml/m^2. Larvae were fed ca. 10 drops of a ground, rabbit-chow, water-suspension prior to the addition of the monomolecular surface film. Beakers were loosely covered with a sheet of clear polyethylene to help prevent evaporation and subsequent loss of ISA-20E on the sides of the beakers. Percentage mortality of each immature stage was recorded at various post-treatment intervals. Tests were conducted in a room maintained at ca. 26.7°C (ambient) and ca. 80% RH.

RESULTS AND DISCUSSION

Results of tests to control natural populations of larvae and pupae of *Ae. taeniorhynchus* with ISA-20E from hand spray applications are presented in Tables 1 and 2. Data obtained at 24 hr post-treatment Table 1, tests 1 and 4–9 indicated that high mortality (98–100%) of immature stages could be achieved in certain salt-marsh/salt water habitats at a spray dosage of 0.45 ml ISA-20E/m² of water surface. In addition, ca. 15–20% of the larvae sampled in test 7 were identified as *Ae. infirmatus* Dyar and Knab, and this *Aedes* sp. was also highly susceptible to ISA-20E at the dosage applied. Helicopter application of ISA-20E (Table 2, test 1) at a surface dosage of ca. 0.37 ml/m² also resulted in 100% mortality of larvae and pupae 24 hr post-treatment. Although high mortality of larvae and pupae was observed at 24 hr post-treatment in 8 trials, results of 5 additional hand sprayer tests indicated that ISA-20E-induced mortality of most larvae of *Ae. taeniorhynchus* (*i.e.* 90% or greater) was delayed to 48 or 72 hr post-treatment (Table 1). Observations at 24 hr post-treatment in 5 additional field trials indicated that very low mortality of larvae (5–20%) had occurred even though persistence of ISA-20E at high film pressure was indicated in each habitat. However, a significant increase in the mortality of immature stages of *Ae. taeniorhynchus* was noted 48 hr post-treatment. High concentrations of living larvae were also present in some of these areas having high film pressure. Even though significant mortality was delayed in these 5 tests, 90% or greater reduction in the natural population of immatures was obtained by 48 or 72 hr post-treatment. Similar results indicating effective but delayed mortality were also observed at 48 and 72 hr post-treatment (Table 2, tests 2 and 3, respectively) in helicopter trials with ISA-20E. The presence of high concentrations of dead larvae and pupae (floating and/or submerged) was always observed in a test site when sampling indicated significant acute or delayed mortality.

Little or no detectable differences in film persistence and percentage mortality of immature *Ae. taeniorhynchus* were noted at the dosages utilized at the termination of field trials (Tables 1–2). However, a time-related sensitivity of *Ae. taeniorhynchus* according to stage of development was noted in these trials, *i.e.* pupae usually exhibited a very noticeable stress response characterized by abdominal flexing within minutes after application of ISA-20E at dosages ranging from 0.30–0.45 ml/m². Mortality of pupae was usually observed 5–7 hr post-application. Comparable levels of larval mortality were not observed until 24–72 hr after treatment even though 3rd and 4th instar larvae were observed to react to the presence of ISA-20E almost immediately after contacting the surface film. They dropped from the surface and formed an "0" shape in the process of trying to remove the film from the opening of their siphons with their mouthparts. Molting of larvae in the presence of ISA-20E was observed in tests 1B and 11–12 (Table 1) and tests 2 and 3 (Table 2). It appears that the molting of larvae to higher instars and larvae to pupae at various intervals during a test may have obscured the mortality data associated with individual instars in some tests. However, some observations indicated that there was a film-induced retardation in the molting sequences of 3rd to 4th instar and 4th instar to pupae when compared to the molting of larvae in untreated areas. In addition, in several tests mortality similar to that produced by certain insect growth regulators was observed.

Bioassays (Table 3) against *Ae. taeniorhynchus* in 400 ml beakers at surface dosages of ca. 0.25 ml ISA-20E/m² indicated that there did not appear to be a

Table 1. Percent reduction of *Aedes taeniorhynchus* larvae and pupae in natural habitats after hand spray application of ISA-20E at 0.45 ml/m².[1]

Test no. (habitat designation)[2]	Pre-treatment mosquito samples: No. larvae by instar and pupae (P); total (T)						Post-treatment													
								No. larvae by instar and pupae						% reduction of larvae by instar and pupae					
	1	2	3	4	P	T	Hr	1	2	3	4	P	T	1	2	3	4	P	T
1(A)	96	303	23	6	0	428	24	0	5	3	0	0	8	100	98.3	87.0	100	—	98.1
(B)	56	1642	4	0	0	1702	24	0	8	8	0	0	16	100	99.5	0*	—	—	99.1
2(A)	0	257	198	111	0	566	48	0	124	101	62	0	287	—	51.8	49.0	44.1	—	49.3
							72	0	2	0	2	0	4	—	99.2	100	98.2	—	99.3
3(C)	0	361	0	0	0	361	48	0	0	0	0	0	0	—	100	—	—	—	100
4(C)	0	212	1395	1691	512	3810	24	0	0	0	0	0	0	—	100	100	100	100	100
5(D)	1	21	39	57	0	118	24	0	0	0	2	0	2	100	100	100	96.5	—	98.3
6(E)	0	0	11	0	0	11	24	0	0	0	0	0	0	—	—	100	—	—	100
7(F)[3]	0	5	1	8	0	14	24	0	0	0	0	0	0	—	100	100	100	—	100
8(G)	50	0	0	0	0	50	24	0	0	0	0	0	0	100	—	—	—	—	100
9(H)	34	240	19	0	0	293	24	0	6	0	0	0	6	100	97.5	100	—	—	98.0
							48	0	0	0	0	0	0	100	100	100	—	—	100
10(I)	0	445	125	3389	91	4050	72	0	0	0	0	12	12	—	100	100	100	86.8	99.7
11(I)	0	88	3276	0	0	3364	48	0	0	65	283	0	348	—	100	98.0	0*	—	89.7
							72	0	0	7	207	0	214	—	—	99.8	0*	—	93.6
							96	0	0	0	169	4	173	—	—	100	0*	0*	94.9
12(I)	0	219	43	2596	0	2858	72	0	0	46	5	10	61	—	100	0*	99.8	0*	97.9
							144	0	0	56	1	5	62	—	100	0*	99.9	0*	97.8

[1] Tests 10–12 were treated at 0.41, 0.33 and 0.30 ml/m², respectively.
[2] Repeat letters indicate re-test of same site.
[3] Mixed population of *Ae. infirmatus*.
* Increase over pre-treatment sample.

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Table 2. Percent reduction of *Aedes taeniorhynchus* larvae and pupae in natural habitats after helicopter spray application of ISA-20E at 0.37 ml/m².

Test no. (habitat designation)[1]	Pre-treatment mosquito samples						Post-treatment												
	No. larvae by instar and pupae (P); total (T)							No. larvae by instar and pupae						% reduction of larvae by instar and pupae					
	1	2	3	4	P	T	Hr	1	2	3	4	P	T	1	2	3	4	P	T
1(J)	0	0	0	97	10	107	24	0	0	0	0	0	0	—	—	—	100	100	100
2(J)	0	12	200	95	0	307	24	0	0	0	140	0	140	—	100	100	0*	—	54.4
							48	0	0	0	15	4	19	—	—	—	84.2	0*	93.8
							72	0	0	0	0	0	0	—	—	—	100	100	100
3(I)	0	2376	1585	0	0	3961	24	0	478	2423	621	0	3522	—	79.9	0*	0*	—	11.1
							48	0	0	203	1756	278	2237	—	100	87.2	0*	0*	43.5
							72	0	0	72	111	0	183	—	—	95.5	0*	100	95.4
							96	0	0	0	0	0	0	—	—	100	100	—	100

[1] Repeat letters indicate re-test of same site.

* Increase over pre-treatment sample.

clearly defined relationship between percentage mortality of larvae according to instar at the various post-treatment intervals. However, actively molting, mixed larval populations seemed to show less acute sensitivity to ISA-20E than a single larval instar. Mortality was seen to increase with exposure time therefore indicating that ISA-20E at this dosage rate could persist in the beakers at least 4 days and kill *Ae. taeniorhynchus* larvae. In general, data generated from field trials concurred with bioassay data and showed no significant correlation between percentage mortality of larvae according to instar at the dosages tested. One hundred percent mortality of *Ae. taeniorhynchus* pupae resulted by 24 hr post-treatment in laboratory tests with ISA-20E at a surface dosage as low as 0.10 ml/m² (Table 3). Field and laboratory observations of mixed populations of pupae and larvae exposed to ISA-20E were consistent.

Observations indicated that ISA-20E is very stable in the field and can be effective in killing mosquito larvae and pupae in some salt-marsh habitats for at least 6 days when applied at a surface dosage as low as 0.30 ml/m² (0.32 gal/acre). In field test 2A (Table 1), ISA-20E at a surface dosage of 0.45 ml/m² persisted for the first 48 hr post-treatment but was not present 72 hr post-treatment. This was attributed to wind gusts and rainstorms which occurred at 24 hr after application. Nevertheless, 99% mortality was recorded at the 72 hr sampling period. With the exception of this test, persistence of ISA-20E was observed at all surface dosages at the termination of all experiments. Water temperature dropped to 14°C at 144 hr post-application in test 12 (Table 1) and was presumed to be responsible for the retarded decay rate of the material. In addition, ISA-20E did not usually persist for longer than 48 hr post-application in sewage treatment systems (Levy et al. 1980). The difference between field stability of ISA-20E applied to salt-marshes and sewage treatment systems was mainly attributed to the microbial and/or chemical degradation of

Table 3. Bioassay of immature stages of *Aedes taeniorhynchus* with ISA-20E at a surface dosage of 0.25 ml/m².

Test no.	Stage (instar)	Cumulative mean percentage mortality of larvae and/or pupae at indicated post-treatment time period (range) 24 hr	48 hr	72 hr	96 hr
1	larval (1st-2nd)	10.0(10.0)	96.7(90.0–100)	96.7(90.0–100)	100
2	larval (1st-2nd)	30.0(20.0–40.0)	50.0(40.0–60.0)	65.0(50.0–80.0)	—[4]
3	larval (2nd)	100	—	—	—
4	larval (2nd)	76.7(60.0–90.0)	100	—	—
5	larval (2nd)	55.0(30.0–95.0)	100	—	—
6	larval (3rd)	100	—	—	—
7	larval (3rd)	90.0(90.0)	95.0(90.0–100)	95.0(90.0–100)	100
8	larval (3rd)	86.7(80.0–90.0)	—	—	—[5]
9	larval (3rd-4th)	3.3(0–10.0)	23.3(0–50.0)	—	66.7[6](50.0–90.0)
10	larval (3rd-4th)	36.7(20.0–50.0)	56.7(50.0–60.0)	66.7(60.0–70.0)	73.3(60.0–80.0)
11	larval (4th)	79.3[1](58.6–100)	100	—	—
12	pupal	100	—	—	—
13	pupal	100[2]	—	—	—
14	pupal	100[3]	—	—	—

[1] Mortality (3.3%(0–10.0%)) corrected by Abbott's formula—No control mortality in other tests.
[2] 100% mortality at 24 hr at a surface dosage of 0.1 ml/m²
[3] 100% mortality at 4.5 hr post-treatment.
[4] 100% mortality at 120 hr post-treatment.
[5] 96.7% (90.0–100%) mortality at 120 hr post-treatment.
[6] 86.7% (80.0–90.0%) and 93.3(90.0–100%) mortality at 120 and 144 hr post-treatment, respectively.

ISA-20E in the sewage and industrial effluent, and the surface fluctuations caused by intermittent pumping and draining sequences used in the sewage treatment process.

Detectable quantities of ISA-20E were not present at each post-treatment sampling location within a test site. This was mainly attributed to wind speed and direction, and to the amount and location of emergent and/or floating vegetation and not to the dosages of ISA-20E that were utilized. Salt-marsh habitats breeding high concentrations of immature *Ae. taeniorhynchus* are usually densely vegetated, and subsequently the areas of water surface treated with ISA-20E are protected from wind-induced translocation and compaction of the film. Irreversible translocation and compaction of ISA-20E can be expected to occur in habitats subjected to relatively strong (16.1 kmph or greater) and unidirectional winds for several days. Observations on the movement of ISA-20E under these adverse treatment conditions have indicated that insufficient area control will usually result. Wind speeds in field tests against *Ae. taeniorhynchus* usually ranged from <3.2–16.1 kmph, however, gusts up to 25.7 kmph were also recorded in one trial.

Hand treatment of densely vegetative habitats was usually by intermittent spraying at various locations within the site and insured even coverage and better film penetration. At the dosages applied, a helicopter, wide fan hand spray, or misting application of ISA-20E over the water surface was observed to produce millions of tiny beads or droplets of excess material. In contrast, much fewer, larger and more localized lenses of excess surface film resulted when a pin-stream spray pattern was used with the hand sprayer. It is presumed that spray application utilizing a wide fan spray pattern would insure a greater particle distribution over the water surface and subsequently reduce the chance that significant concentrations of localized lenses of floating ISA-20E would be lost due to wind-induced translocation and/or drying of a habitat in various locations. This technique would also reduce loss of material from possible adherence to floating or emergent vegetation and other organic material.

One helicopter evaluation was conducted strictly to determine the efficacy of aerial penetration of ISA-20E at a dosage of 0.4 gal/acre in a highly vegetated salt-marsh habitat containing no immature mosquitoes. The wind speed at the time of aerial application was 3.2 kmph. The water surface of this habitat was not continuous but was confined to numerous potholes and low lying areas containing dense concentrations of pickerelweed. Samples showed that material was present in ca. 90% of the treatment area immediately after application but that degradation over the entire habitat had occurred within 24 hr post-treatment. Therefore, a sufficient quantity of the total dosage (0.37 ml/m^2) did not penetrate the vegetation to provide the film pressure and stability necessary for effective mosquito control. It should be noted that helicopter application of ISA-20E at a dosage of ca. 0.40 gal/acre resulted in satisfactory penetration of film through a moderately vegetated mangrove habitat mainly characterized by dense areas of emergent cenicella (Table 2, test 3).

Recent data (Levy et al. unpublished) have indicated that ISA-20E can be suspended in water with agitation at recommended dosages for mosquito control and therefore be applied at a rate of 5–7 gal/acre to facilitate penetration through heavily vegetated areas. Results of preliminary field tests with a hand-held compressed air sprayer with a water-based ISA-20E formulation at ca. 5 gal/acre (0.35 gal ISA-20E/acre) against larvae and pupae of *Ae. taeniorhynchus* and *Ps. columbiae* have indicated that there were no significant differences in percentage mortality of immatures and field persistence between ISA-20E applied alone or as a water suspension (Levy et al. unpublished). Additional data (Levy et al. unpublished) indicated that spray treatment of mosquito habitats at pressures of 100–300 psi with a trailer mounted spray system greatly increased penetration of ISA-20E into densely vegetated areas and assured better coverage and subsequent control of immature mosquitoes.

INFLUENCE OF ENVIRONMENTAL FACTORS. Observations in salt-marshes 24 hr after treatment indicated that ISA-20E was usually present in areas that were far removed from the initial points of hand spray application. Thus information was obtained about the effective lateral spreading pressure of this monomolecular film when applied at little or no spray pressure. In moderate to high winds (12.9 kmph or greater) initial hand spray application of ISA-20E was at the upwind portion of a habitat. This procedure allowed the wind-pushed film to translocate larvae and pupae to the downwind portion of a habitat where increased film and population pressures could maximize mortality. Even though displacement of ISA-20E over the water surface was significant under certain wind conditions, redistribution of the film was observed when the wind speed decreased or the wind direction shifted. These observations were also reported by Levy et al. (1980) in their spray tests with ISA-20E against *Culex* spp. larvae and pupae in an industrial sewage treatment plant.

Rain (ca. 1 cm) was recorded at 24 hr post-treatment in test 2A (Table 1) with a slight increase in water surface area of the original habitat, and little or no mortality was observed. Nevertheless, the dosage of ISA-20E utilized (0.45 ml/m^2) was sufficient to cover the new areas with film for 48 hr or longer and kill 99% of the larvae and pupae at 72 hr post-treatment, even though effective film pressure was not evident 72 hr after treatment. Again, a high film pressure and 100% mortality was observed during test 3 (Table 2) following a 1.3 cm rain 96 hr after treatment. Similarly, rain had little effect on the action of ISA-20E on *Culex* mosquitoes breeding in sewage treatment systems (Levy et al. 1980).

It is presumed that utilizing high dosages of ISA-20E (>0.45 ml/m²) that would provide lenses of excess material can be an effective means of controlling a new brood of larvae in newly flooded areas adjacent to a treatment site. Therefore, an overdosing technique could be utilized in selected mosquito habitats that are known to increase in size as a result of tidal fluctuation or persistent rain. In this way, effective film pressure and uniform coverage could be expected over the entire habitat.

Reiter (1978) reported that levels of dissolved oxygen in a mosquito habitat were important in evaluating larvicidal approaches which depend on suffocation. He indicated that effective levels of larval control were most likely to be expected with a monomolecular surface film when the dissolved oxygen of the water was less than 30% saturated, the exact value depending on the mosquito species. It is presumed that prolonged survival of larvae that were prevented from normally penetrating the water interface to obtain atmospheric oxygen by a surface film such as ISA-20E was via cuticular respiration and air storage within the tracheal system. Dissolved oxygen levels of 2.8, 1.2, 0.9, 1.1, and 0.7 ppm were recorded at pre-treatment, 24, 48, 72, and 96 hr post-treatment, respectively during test 3 (Table 2). Perhaps a factor accounting for the acute sensitivity of pupae to ISA-20E was their presumed inability to carry on cuticular respiration. Dissolved oxygen levels of 0.6, 0.6, 0.7, and 3.2 ppm were recorded before treatment in tests 1A and B, 4, and 12, respectively. Total percentage mortality of larvae and pupae at 24 hr post-treatment in tests 1A and B and 4 was 98.1, 99.1 and 100, respectively (Table 1). Substantially less mortality of larvae (<10%) was observed at 24 hr post-treatment in test 12 (Table 1) thus indicating a significant negative correlation between the acute or delayed larvicidal action of ISA-20E on larvae of *Ae. taeniorhynchus* and the dissolved oxygen concentration of the mosquito habitat. Similar results were obtained in test 3 (Table 2).

High mortality (ca. 90%) of larvae and pupae of *Culex* spp. was usually obtained at 24 hr post-treatment in sewage treatment systems (Levy et al. 1980) having dissolved oxygen concentrations of 0.1–0.3 ppm. However, ISA-20E-induced mortality of larvae of *Cx. quinquefasciatus* of 90% or greater was not obtained until 96 hr post-treatment in one test in a sewage treatment polishing pond having a dissolved oxygen concentration of 1.2 ppm (Levy et al. unpublished). High acute mortality of larvae of *Cx. quinquefasciatus* exposed to ISA-20E was not obtained in laboratory bioassays conducted in highly oxygenated fresh water, further indicating the relationship between the amount of dissolved oxygen and the effectiveness of a monomolecular film.

Temperature and salinity are factors that have an effect on the solubility of oxygen in water (Spotte 1979). Temperature and the solubility of oxygen are inversely related. In addition, a rise of 10°C will cause the rate of oxygen uptake in a poikilothermic organism such as a mosquito larva to double or triple. The rate of development would also be proportionally increased. Reiter (1978) has presented data on the survival times of larvae at different temperatures. He showed that at a low concentration of dissolved oxygen larvae could survive longer at lower temperatures, presumably due to the reduced rate of respiration. Furthermore, he showed that at 32 C° the critical level of dissolved oxygen was about 50% higher than at 27°C. Pre- and post-treatment water temperatures for all *Ae. taeniorhynchus* habitats ranged from 14–38°C. Furthermore, temperature is inversely related to surface tension (Anonymous 1980).

Salinity is also inversely related to the concentration of dissolved oxygen in a mosquito habitat (Spotte 1979). An increase in salinity would cause a decrease in the amount of dissolved oxygen. For example, the salinity in test 3 (Table 2) fluctuated from 8.0–15.0 ppt over the 96 hr test period. Salinity in all test sites ranged from 3.5–22 ppt at pre-treatment.

Furthermore, the concentration of free carbon dioxide in the water is a function of pH (Spotte 1979). As the pH decreases there is an increase in the concentration of carbon dioxide. It should be noted that respiration and photosynthesis, a function of the amounts of plants and animals in a mosquito habitat, affect the pH of the water, *i.e.* respiration would cause the pH to decline while photosynthesis produces a pH increase. *Ae. taeniorhynchus* test sites were observed to range in pH from 6.7–8.3 at pre-treatment.

Within and between species differences in the tolerance of mosquito larvae to reduced levels of oxygen were presumed also to affect the acute mortality induced by ISA-20E. Field and laboratory bioassay observations of *Ae. taeniorhynchus* larvae exposed to ISA-20E indicated that there was a differential sensitivity or survival rate of larvae of the same instar over the 24 hr observation periods. Reiter (1978) has indicated that *Cx. quinquefasciatus*, a mosquito typically found in polluted habitats having low dissolved oxygen, had cuticular adaptations that allowed it to tolerate significantly lower concentrations of dissolved oxygen than 2 *Anopheles* spp. Laboratory tests at a dosage of 0.25 ml/m^2 have shown that *Cx. quinquefasciatus* assayed in highly oxygenated fresh water was significantly less sensitive to ISA-20E than was *Ae. taeniorhynchus* of the same instar that were tested in 50% sea water (Levy et al. unpublished). Fourth instar larvae of *Ae. taeniorhynchus* have been observed to contact vigorously the surface film in repeated attempts to penetrate through the film with their siphons at various angles to obtain atmospheric oxygen. These observations indicate that many of these angular penetrations are successful since some larvae in film-treated beakers seem to be hanging from the surface in a manner comparable to larvae in controls, thereby presumably obtaining atmospheric oxygen and prolonging survival. Furthermore, laboratory observations (Table 3) of mixed instars of *Ae. taeniorhynchus* larvae exposed to ISA-20E have suggested that prolonged survival may be partially attributed to molting at various intervals during an experiment. It is presumed that this process would remove internal and external portions of the respiratory siphon that were plugged or coated with the monomolecular film and allow larvae to attempt to repenetrate the surface film to obtain atmospheric oxygen. Therefore, the age of larvae within an instar could be important in evaluating larvicidal effects induced by a monomolecular organic surface film such as ISA-20E.

Qualitative data on non-target animals and plants (Table 4) exposed to various dosages of ISA-20E during field trials to control mosquitoes have indicated that this film will cause little or no adverse effects to the environment. Some mortality of pupae and/or emerging adults of certain midge species (Chironomidae) breeding in aeration and decomposition ponds at sewage treatment systems was noted; however, significant mortality of midges was also observed in some control (untreated) sewage ponds containing a similar layer of natural surface scums. Therefore, the true impact of ISA-20E on the reduction of the midge population is not known. Adult dragonflies were observed to oviposit in water treated with ISA-20E and a *Gambusia* sp. was observed eating large lenses of floating ISA-20E with no apparent adverse affects. Field and laboratory tests indicated that predation and asexual reproduction of the mosquito planarian *Dugesia dorotocephala* (Woodworth) and the infectivity and development of the mosquito nematode *Romanomermis culicivorax* Ross and Smith were not adversely affected by ISA-20E at surface dosages of 0.4–0.5 ml/m^2. Therefore, this monomolecular film may be employed in integrated mosquito control programs. Furthermore, no mortality, defoliation or discoloration of vegetation was observed in habitats treated with

Table 4. Non-target organisms observed in natural habitats before and after experimental application of ISA-20E to control immature mosquitoes.

Animals			Plants
		Amphibians Reptiles Fish Others	Algae Emersed Submersed Floating
Insects	Crustaceans		
Baetidae	Astacidae	Ranidae	Characeae (*Chara* spp.)
Libellulidae	Notostraca	Trionychidae (*Trionyx ferox*)	Typhaceae (*Typha* spp.)
Aeschnidae	Conchostraca	Emydidae	Poaceae (Graminae)
Gomphidae	Daphnidae	Alligatoridae (*Alligator mississippiensis*)	Lemnaceae (*Lemna minor, Spirodela polyrhiza*)
Coenagrionidae	Astacidae	Colubridae (*Natrix* spp.)	Verbenaceae (*Lippia lanceolata*)
Corixidae	Isopoda	Poeciliidae (*Gambusia* sp.)	Avicenniaceae (*Avicennia germinans, Rhizophora mangle, Laguncularia racemosa*)
Notonectidae		Vorticellidae	Aizoaceae (*Sesuvium portulacastrum*)
Nepidae		Amoebidae	Anacardiaceae (*Schinus terebinthefolius*)
Belostomatidae		Planariidae (*Dugesia dorotocephala*)	Schrophulariaceae (*Micranthemum umbrosum*)
Dytiscidae		Mermithidae (*Romanomermis culicivorax*)	Umbelliferae (*Hydrocotyle umbellata*)
Gyrinidae		Tubificidae	Pontederiaceae (*Eichhornia crassipes*)
Hydrophilidae		Hydrachnidae	
Psychodidae			
Chironomidae			
Syrphidae			

ISA-20E when compared to controls. Although no quantitative data were obtained concerning the effects of ISA-20E on the natural populations of animals and plants (Table 4), general observations indicated that there appeared to be no increased mortality of non-target organism when compared to untreated or control areas. Furthermore, no acute or chronic skin or eye irritation or respiratory effects were reported by spray applicators exposed to ISA-20E during experimental applications. ISA-20E has an acute oral LD_{50} for rats of 20,000 mg/kg, and has been classified as non-toxic orally and non-irritating to the eyes and skin (Reynolds, personal communication).

In summary, our data indicated that ISA-20E can be sprayed by conventional ground and aerial systems at surface dosages of 0.32–0.48 gal/acre and control larvae and pupae of *Ae. taeniorhynchus* in natural habitats varying in water qualities and climatological conditions without producing adverse health and environmental effects. However, at the dosage applied, aerial application of ISA-20E in densely vegetative salt-marsh habitats with the spray system utilized is expected to result in little or no penetration of material through the canopy. In addition, strong and persistent unidirectional winds can significantly reduce the mosquito control effectiveness of this monomolecular organic surface film. New and more effective solid and liquid formulations of ISA-20E have been developed and are presently being evaluated under a variety of field conditions.

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JAMES B. COULTER
SECRETARY

LOUIS N. PHIPPS, JR.
DEPUTY SECRETARY

STATE OF MARYLAND
DEPARTMENT OF NATURAL RESOURCES
TIDEWATER ADMINISTRATION
TAWES STATE OFFICE BUILDING
ANNAPOLIS 21401
(301) 269-2784

June 30, 1982

Mr. Robert H. Forste
Asst. Scientific Advisor
Research Division
Legislative Reference Service
Room 110
90 State Circle
Annapolis, Maryland 21401

Dear Mr. Forste: Bob

In June, the Environmental Matters Committee addressed the topic of mosquito control, potential management techniques and their effect upon wetlands. One particular technique that was mentioned concerned the use of Monomolecular films the effectiveness of which has been presented in several articles which I have enclosed for your perusal.

Being curious as to the applicability of this technique to Maryland as an additional alternative to Open Marsh Water Management, I asked Mr. Tom Dolan, a systems ecologist on my staff to examine the potential of this technique. His memo to me on the subject is attached. Basically, while Tom believes the technique to be effective where tested, he states that several shortcomings exist however:

1) In the articles, the long range ecological and water quality impacts of the film were not evaluated. Almost no mention was made of the film's effect on other species; only the target species were mentioned.

2) Aerial applications of the film were found to be unsatisfactory in highly negatated salt marsh habitat. The principal breeding areas on the Eastern Shore are typically densely vegetated with marsh species and aerial application might be ineffective there also.

TTY FOR DEAF — BALTIMORE 269-2609. WASHINGTON METRO 565-0450

3) Little information was provided concerning the duration of the film on a natural water body and no information was given concerning the ultimate fate of the film (its breakdown components), and

4) No analysis was provided as to the costs of application.

I thought that this would be useful to you in your work with the General Assembly.

Sincerely,

Sarah

Sarah J. Taylor, Ph.D.
Director, Coastal
Resources Division

SJT:rrc

Enclosures

JAMES B. COULTER
SECRETARY

LOUIS N. PHIPPS, JR.
DEPUTY SECRETARY

STATE OF MARYLAND
DEPARTMENT OF NATURAL RESOURCES
TIDEWATER ADMINISTRATION
TAWES STATE OFFICE BUILDING
ANNAPOLIS 21401 (301) 269-2784

June 30, 1982

MEMORANDUM

TO: Sarah Taylor

FROM: Tom Dolan

SUBJECT: Mosquito Control by Monomolecular Films.

The following comments are based on the enclosed set of articles.

Monomolecular films can be formed by applying certain petroleum or alcohol based compounds to isolated bodies of water. The lifetime of monomolecular films appears to be very contingent upon the size of the water body, vegetation types and exposure to rain and wind. The lifetime of the films apparently is on the order of several days.

Monomolecular films can control mosquite populations through two primary mechanisms:

1) Reduction in water surface tension. Mosquito pupae and larvae may not be able to orient property at or near the surface and therefore suffocate. Egg-cases may sink and adults may have difficulty when emerging.

2) Reduction in the respiratory system's efficiency of larvae and pupae. The parent compound may 'wet' the mosquito's trachae and cause death by suffocation.

The journal articles presented a very strong case for the effectiveness of monomolecular films in controlling mosquitoes. The March '82 Mosquito News article reported that over 90% control of larvae and pupae was achieved during one testing period. Laboratory tests also indicated that adult mosquitoes can drown and eggs can sink in the monomolecular film.

TTY FOR DEAF — BALTIMORE 269-2609, WASHINGTON METRO 565-0450

The results of these studies were very encouraging in so far as mosquito control was concerned. However, I can not recommend adoption of this technique for use in the Chesapeake Bay region. A number of issues were either not addressed or not addressed sufficiently in the articles.

1) Effect on Non-Target Species. The articles dealt solely with the effect of monomolecular films on mosquitoes. Almost no mention was made of the film's effect on other species. The monomolecular films would probably disrupt the life cycle of any species which ultilizes the air-water interface. There could be a significant negative long term ecological impact.

2) Problems Associated with Application the June '81, Mosquito News article stated that aerial application of the film was not satisfactory in a highly vegetated salt-marsh habitat. The prime mosquito breeding areas on the Eastern Shore are typically densely vegetated with marsh species. Aerial application on the Eastern Shore might not be possible.

3) Lifetime and Degradation of Film. Little information was provided concerning the duration of a film on a natural water body. But more importantly, no information was given concerning the ultimate fate of the film. How long does it take to degrade and what are the breakdown products? In addition, one of the more highly touted compounds, isostearyl alcohol (ISA-20E), is apparently non-toxic to humans. It is used commercially by the cosmetic industry. However, nothing apparently is known of its possible negative impact on aquatic organisms.

4) Economics of Monomolecular Films. No analysis was provided of the costs of the monomolecular compounds versus more traditional mosquito agents. Use of Monomolecular films may be exorbitantly expensive.

Summary

Monomolecular films control mosquitoes principally by disrupting the physical characteristics of the air/water interface. Because of the film's mode of action, mosquitoes would probably not become resistant over time as they do with traditional chemical agents. Monomolecular films proved very effective in controlling mosquitoes in sewage treatment ponds and certain natural habitats. However, the long range ecological and water quality impacts of the film have not been evaluated. Before such films can be used in the Chesapeake region, additional studies will also have to focus on the film's costs, techniques of application and its lifetime and degradation in natural (E. Shore) environments.

TD/rrc

Harry Hughes
Governor

Wayne A. Cawley, Jr.
Secretary
Hugh E. Binks
Deputy Secretary

STATE OF MARYLAND
DEPARTMENT OF AGRICULTURE
Parole Plaza Office Building
Annapolis, Maryland 21401

June 8, 1982

Mr. Robert K. Lloyd
Administrative Assistant
County Commissioners of Dorchester County
County Office Building
P.O. Box 26
Cambridge, Maryland 21613

Dear Mr. Lloyd:

Use of the mosquito larvicide, Abate^{R}, has been under question by certain groups in Maryland for the past two years. This Department has been attempting to investigate all claims and concerns objectively and scientifically. We consider the mosquito control activities in Dorchester County to be a cooperative effort and want to assure you that the County Commissioners will be advised and consulted as evidence is made available either for or against the use of Abate.

Because this product has been under study approximately 15 years, there is a large volume of data available, but critics occasionally raise questions. This Department is committed to making decisions on the use of pesticides on the basis of available scientific data. In good conscience, we cannot advocate new techniques that will greatly increase the cost of mosquito larviciding unless there is solid scientific evidence to substantiate a change.

At this time, the principal complaint against Abate appears to be from the muskrat trappers who feel that the decline in numbers of muskrats in some marshes is due to mosquito spraying with Abate. The Department has taken this charge very seriously and has been investigating. The Mosquito Control Section has accumulated much pertinent information from diverse sources. A recent (August, 1981) publication by the Environmental Protection Agency (EPA) entitled, "Temephos (Abate^{R}) - Pesticide Registration Standard", reviews research articles and publications relating to Abate^{R}. The section on Toxicology presents no new or alarming data that would cause us to suggest that anyone stop using Abate. This publication contains several hundred references which EPA evaluated in a review prior to approving Temephos (Abate) registration.

Correspondence with Dr. Lucille Stickel, Director, Patuxent Wildlife Research Center, (letter dated Aug. 7, 1980) states "we consider it unlikely that Abate is the cause of the muskrat problem (death)". You may already have this letter, but a copy is enclosed.

Phone: 301-269-2161

TELEX No. 87856

Mr. Robert K. Lloyd

Correspondence with Don R. Perkuchin, Bombay Hook, Delaware, indicates that, "We do not believe that the chemical Abate adversely affects muskrat population or any other mammals located in the treated area". Amounts and application techniques for Abate are the same in Maryland as in Delaware. Abate applications have been permitted on other Federal Refuges in the Northeastern United States and there is no documented incident of bird, mammals, or other desirable life forms being killed as a result of Abate use.

While there is much additional evidence supporting the safety of Abate to mammals, fishes, invertebrates and birds, the reports implicating this chemical as being deleterious to non-target species have been largely circumstantial and vague. Trappers have suggested that reduced muskrat populations in some marshes are associated with aerial application of Abate, yet spraying with Abate had not occurred in several areas where damage was claimed. When Patuxent researchers met in 1980 with muskrat trappers, the symptoms described by the trappers for some muskrats could not be related to known pesticide poisoning symptoms. To date, efforts to collect and submit any type specimens to Patuxent for analysis have not been successful. MDA employees spend many hours on the marshes following Abate spraying to assay spray effectiveness, yet moribund or dead specimens (muskrats, birds, fish or crabs) have not been observed.

Abate is the most effective control, the most cost-effective chemical, and the least toxic chemical larvicide available for mosquito control. The Department does not promote the use of any particular chemical, except for the reasons stated above and toxicity to non-target species is a primary concern. At this time, there is no other pesticide available for aerial application to large acreages (up to 20,000 acres) that is competitive with Abate when all use factors are considered.

The implications of discontinuing the use of Abate are most significant. There are alternative products that may produce satisfactory control, but the increased costs will be a real problem. While Abate spraying is costing $1.53/acre in 1982, other larviciding products such as Bacillus thuringiensis israelensis (BTI) would cost $9.00/acre and monomolecular surface films may cost $9.00 to $11.00/acre. Obviously, we would not decide to use such costly techniques without the support of the cooperating county.

I want to thank you and the Dorchester County Commissioners for your continued support and cooperation.

Sincerely,

Wayne A. Cawley, Jr.
Secretary

WAC:sd
Enc.

MARYLAND
DEPARTMENT OF AGRICULTURE

Harry Hughes, Governor
Wayne A. Cawley, Jr., Secretary
[illegible], Deputy Secretary
Hugh E. Binks,

Parole Plaza Office Building, Annapolis, Maryland 21401
Phone: 301—269-2161

OFFICE OF PLANT INDUSTRIES AND PEST MANAGEMENT
MOSQUITO CONTROL SECTION

June 8, 1982

Mosquito Control Study Committee
90 State Circle
Annapolis, Maryland 21401
ATTN: Dr. Robert Forste

Dear Committee Members:

Enclosed is information on aerial spraying for mosquito control in Maryland. The acreage figures represent repetitive spraying of selected mosquito breeding marshes. Some high salt marshes are sprayed six or more times each year as individual broods of salt marsh mosquitoes develop. These figures do not include spraying with ground equipment which is usually concentrated around populated areas.

Dibrom 14 is applied at 1.0 fluid ounces per acre and Abate 4E at 1.5 fluid ounces per acre.

The Mosquito Control Section staff has compiled data on mosquito breeding salt marshes as follows:

Dorchester County: 17,080 acres have been documented as mosquito breeding areas out of 62,850 acres surveyed.

Somerset County: 8,700 acres of documented breeding marsh.

Wicomico County: 1,350 acres of documented breeding marsh.

Worcester County: 2,000 acres of documented breeding marsh as yet not brought under water management.

MDA has Corps of Engineers permits to perform open marsh water management on some of these areas, and we are attempting to perform as much permanent ditching work as is possible with existing equipment, people and funds.

Also enclosed, please find copies of studies done on muskrats in several areas of the U.S., including Dorchester County. These studies document quite well the severe fluctuations that occur in muskrat populations as a result of weather, disease, predators, salinity changes, and other factors. The letter by Dr. Lucille Stickel indicates our earlier efforts to resolve the concerns of the trappers with regard to Abate spraying.

Abate is also applied to Delaware marshes, including the Federal Refuge at Bombay Hook. The letter from Mr. Don R. Perkuchin, Refuge Manager, indicates that there has not been a problem with muskrats on Abate sprayed marshes at this refuge over the past 11 years. The Delaware mosquito control program is applying Abate 4E by air in a manner nearly identical to the Maryland program.

Sincerely,

Stanley R. Joseph

Stanley R. Joseph, Chief
Mosquito Control Section

SRJ:sd

Enc.

United States Department of the Interior

FISH AND WILDLIFE SERVICE
PATUXENT WILDLIFE RESEARCH CENTER
LAUREL, MARYLAND 20811

June 17, 1982

Dr. Robert Forste
Deputy Chief
Research Division
Maryland General Assembly
90 State Circle
Annapolis, MD 21401

Dear Dr. Forste:

Attached are copies of the correspondence reporting the results of our studies on abate. The data were released through an irregular and circuitous route, but the correspondence does summarize all the information available at this time.

The results on the mallard study should be available soon. As I understand it, some statistical problems are now being worked out. We will share this information with you and Dr. Joseph as soon as it has completed the review process.

Sincerely yours,

Russell J. Hall

Russell J. Hall
Assistant Director

TELEPHONE—AREA CODE 301 776-4880 (MARYLAND EXCHANGE)
TELEGRAMS—FISH AND WILDLIFE SERVICE, WASHINGTON, D.C. 20240

Russ -
Here is the Abate stuff.
Don

Harry Hughes, Governor
Wayne A. Cawley, Jr., Secretary
Hugh E. Binks, Deputy Secretary

MARYLAND

Parole Plaza Office Building, Annapolis, Maryland 21401
Phone: 301-269-~~2161~~ 2961

DEPARTMENT OF AGRICULTURE OFFICE OF PLANT INDUSTRIES AND PEST MANAGEMENT
MOSQUITO CONTROL SECTION

March 22, 1982

Dr. Christian E. Grue
U.S. Department of the Interior
Fish and Wildlife Service
Patuxent Wildlife Research Center
Laurel, Maryland 20811

Dear Dr. Grue:

Thank you for your recent letter and the attached reports on Abate toxicity and residue analysis. I am not surprised that only low amounts of Abate residue were detected, since our application rate is only 1.5 fluid ounce of Abate R 4E per acre. This is .047 pounds of active ingredient per acre which is well below the label rates for the same chemical when applied as a granular formulation (.1 pound to .2 pound active ingredient per acre). The rate approved for citrus referred to in Dr. David Hoffman's memorandum appears to be 21 times that being used on Maryland salt marshes.

When most aerial spray data are analyzed for swath evenness, swath overlap, faulty nozzles or other operation problems, it would be highly unlikely that label rates could ever exceed the desired rate of .047 lbs./acre by more than a factor of 2 to 4 fold, even if swaths became superimposed due to pilot error. How does one extrapolate one pound/acre data to .047 pounds per acre application rate used in the Maryland areas at this time?

I appreciate your interest in our program and the assistance we have had from Dr. David Hoffman, Dr. Lucille Stikel and other staff members of the Fish and Wildlife Service. Thank you again for your assistance.

Sincerely,

Stanley R. Joseph

Stanley R. Joseph, Chief
Mosquito Control Section

SRJ:sd

c.c. Cyrus R. Lesser

March 4, 1982

Dr. Stan Joseph
Maryland Department of Agriculture
Mosquito Control Section
Parole Plaza
Parole, Maryland 21401

Dear Dr. Joseph:

On February 17, Jim Fleming and I met with Cyrus Lesser to discuss the possibility of studying the effects of Abate® on birds within the salt marshes surrounding Fishing Bay. Mr. Lesser was most cooperative and provided us with valuable information. Unfortunately, Jim and I may not be able to pursue the study in 1982 because of time constraints.

During our conversation, Cyrus mentioned that neither you or he had heard from Patuxent concerning the results of previous Abate® studies for which you had provided samples or assisted in their collection. I told Cyrus that I would check into this and see that the information was made available to you.

Attached please find summaries of research findings to date provided by the two principal investigators, Drs. Hoffman and Franson. We apologize for the delay in providing this information to you.

If you have any questions concerning the studies, please contact Drs. Hoffman and Franson.

Sincerely,

Christian E. Grue
Research Biologist

Attachments

cc: C. Lesser

GRUE:lmt

March 3, 1982

Physiologist, Environmental Physiology and Toxicology Section

Testing of Abate for toxicity on mallard and bobwhite eggs

Christian E. Grue, Research Biologist, Population Ecology Section

The effects of Abate[R] ('abate 4 E', the emulsifiable concentrate) were examined on mallard and bobwhite embryo development. Eggs were treated externally at 3 days of incubation using this formulation in a water vehicle and also in a non-toxic oil vehicle (a mixture of aliphatic hydrocarbons) to screen for possible toxic effects of spray applications. We based our doses on multiples of the maximum permissable field level of application which is 1 lb/100 gal/acre according to the EPA compendium (used on citrus crops). At 5 times the maximum field level of application, there were no significant effects on mortality and growth, or any teratogenesis. Even doses at 20 times the field level of application caused minimal mortality and teratogenesis.

David J. Hoffman

GRUE:lmt

UNITED STATES GOVERNMENT

memorandum

DATE: February 25, 1982

REPLY TO ATTN OF: Research Veterinarian

SUBJECT: ABATE® Residues in Grasses; ABATE® Reproductive Study

TO: Christian E. Grue
Research Biologist

Attached are copies of memoranda and residue analyses concerning samples of Scirpus olneyi and Spartina alternafolia collected near Blackwater National Wildlife Refuge on 21 September 1980. Of 11 samples submitted, ABATE® was detected in 2 S. alternafolia samples (<1 μg/g, wet wt). The ABATE® concentration and frequency of detection is somewhat low compared with literature indicating an average concentration of 1 ppm in grasses after application of granular ABATE®. ABATE® sulfone was detected in none of the samples, and analysis for ABATE® sulfoxide was not done because a suitable standard was not available.

The data from our mallard reproductive study are awaiting final statistical analysis and interpretation, so all results must be considered preliminary. However, the data indicate that percentage survival of ducklings to 21 days of age was reduced in both treatment groups when compared with controls. Plasma cholinesterase activity was apparently reduced by ABATE® exposure in 21-day-old ducklings, but brain cholinesterase was not affected.

Chris

J. Christian Franson

APPENDIX B

Reprinted from the Archives of Environmental Health
Feb. 1967, Vol. 14, pp. 283-288

Toxicology of Abate in Laboratory Animals

Thomas B. Gaines, BA; Renate Kimbrough, MD; and Edward R. Laws, Jr., MD, Atlanta

As THE NEED for a chemical compound to control the breeding of *Aedes aegypti* mosquitoes in drinking water became essential to the success of the *A aegypti* eradication program of the Public Health Service Communicable Disease Center, a series of compounds was tested for biological effectiveness against larvae of *A aegypti* at the Communicable Disease Center laboratories in Savannah, Ga. One compound, Abate (O,O,O′,O′-tetramethyl O,O′-thiodi-*p*-phenylene phosphorothioate), showed a combination of remarkable effectiveness as a larvicide when added to water at the rate of 1 ppm and low mammalian toxicity in initial studies. The formulation used to treat water actually releases approximately 0.5 ppm soon after treament, and that concentration gradually decreases with time. A series of toxicological investigations in laboratory animals was planned and executed for Abate, and the results are reported here.

Materials and Methods

All of the Abate used in these experiments (except the tests on dogs) was technical material. Three separate manufacturing batches of technical Abate designated A, B, and C were examined, but the first was studied more thoroughly, and it is the one referred to in this report unless another is specified. The material given to dogs was 43% emulsifiable spray concentrate.

Submitted for publication Aug 8, 1966; accepted Aug 23.

From the Toxicology Section, Technology Branch, Communicable Disease Center, Public Health Service, US Department of Health, Education, and Welfare, Atlanta. Dr. Laws is now at Johns Hopkins Hospital, Baltimore.

Reprint requests to Communicable Disease Center, Atlanta 30333 (Mr. Gaines).

Both young and adult Sherman-strain rats, adult white mice, weanling white rabbits, adult guinea pigs, adult dogs, adult white leghorn hens, chicks, and ducklings were utilized. The mammals were obtained from production facilities in the Communicable Disease Center; the birds were obtained from commercial sources. All the animals were caged individually except that some which were fed Abate in their diet were caged in groups.

For oral lethal dose (LD_{50}) studies, the technical grade compound was given by stomach tube without dilution. The same material was used for dermal toxicity studies, and the rats were confined in restraining cages so they could not lick themselves. All LD_{50} values were calculated by the method of Litchfield and Wilcoxon.[1]

To explore the possibility of potentiation in toxicity when Abate and malathion are given together, groups of two adult female rats each were given both compounds in the following proportions of the LD_{50} value of each: 1/2, 1/4, 1/8, and 1/16.

Details of dosages used in various studies of subacute toxicity are shown in Table 1. For repeated oral administration by stomach tube, technical Abate was mixed with peanut oil in varying concentrations to provide a standard dosage volume of 0.005 ml/gm of body weight. For the studies where Abate was placed in a diet to be taken ad libitum, technical Abate was mixed with a small amount of standard feed, and the concentration was adjusted by dilution. Complete details of the technique of dietary formulation are given by Gaines and Kimbrough.[2] Dosages were determined by food consumption measurements.

When Abate was administered in drinking water, the solutions were prepared fresh every 24 hours. In collecting samples from the drinking pans for chemical analysis, an effort was made to avoid water near the bottom of each pan and to take water near the surface, which a dog might lap up and drink. The analyses indicated that four hours after preparation of the solution, there was 7.1 to 8.7 ppm of Abate in the 10-ppm watering pans and 45.1 to 52.0 ppm in the 50-ppm pans. After 24 hours there was 5.3 to 7.1 ppm in the 10-ppm pans and 41.0 to 51.9 ppm in the 50-ppm pans. The intake was approximately 0.6 to 0.8 mg/kg/day and 3 to 4 mg/kg/day at the two dosage levels.

In studies of the possible effects of Abate on reproduction, male and female rats were started on diets containing 500 ppm of Abate (approximately 25 mg/kg/day) at the time they were placed together for breeding. This dosage was sufficient to cause symptoms of poisoning in some of the rats. Dosage was maintained throughout mating, gestation, parturition, and lactation. The animals were kept under standard colony conditions and allowed to reproduce normally.

Autopsies were performed on representative animals from each phase of the investigations; microscopic studies were included wherever indicated.

Plasma and red blood cell (RBC) cholinesterase values were determined by automated potentiometric titration at constant pH, a modification of the method described by Jensen-Holm et al.[3] Blood for this test was taken by cardiac puncture (rats) or from the median artery of the ear (rabbits) or the cephalic vein of the leg (dogs).

Results

The results for each experiment in the series are discussed individually below. In the cases were Abate produced cholinesterase depression, the RBC enzyme was always affected more rapidly and to a greater extent than the plasma enzyme; therefore, these results are presented primarily in terms of effect on RBC cholinesterase.

Acute Toxicity to White Rats and Mice.—The results are summarized in Table 2. Both male and female rats showed definite, typical signs of organic phosphorus poisoning when given single doses by stomach tube at rates as low as 750 and 500 mg/kg, respectively, although the lowest lethal dosages were much higher. Batch A was definitely less toxic than batches B and C; however, the smallest dosage of any batch that killed a rat was 4,000 mg/kg.

The acute oral LD_{50} was determined on male mice using the same technique as for the rats. The LD_{50} was 4,700 mg/kg, and the lowest lethal dose was 3,000 mg/kg.

The highest dermal dosage of Abate that is practical to apply accurately to rats is 4,000 mg/kg. This level killed two of ten rats but

Table 1.—*Dosages of Abate Used in Subacute Studies**

Species	Sex	Route and Medium		Dosage Range (mg/kg/day)	Duration† (days)
Rat	M	ST‡—peanut oil		0, 1, 10, 100	44
	M	ST—peanut oil		0, 1, 10	28
	M	Diet—regular food			
		conc (ppm):	0	0	99
			2	0.24-0.12§‖	99
			20	2.4-1.2§‖	99
			200	24-11§‖	99
			2,000	150§	99
Rabbit	M	ST—peanut oil		0.1, 1, 10	35
	M	ST—peanut oil		100	5
Guinea pig	M	ST—peanut oil		100	5
Dog	Both	Diet—water			
		conc (ppm):	0	0	129
			10	0.6-0.8§	129
			50	3-4§	129
Chicken	F	Diet—regular food			
		conc (ppm):	0	0	108
			250	7.4§	108
			500	15.3§	108
			1,000		<44
			2,000		<44
Chick	Both	ST—peanut oil		10, 25, 50	5
Duckling	Both	ST—peanut oil		10, 25, 50	5

* The number of animals used in each test is shown in Table 3.
† Duration of dosage. At high dosage levels some animals died before the test was complete.
‡ ST indicates stomach tube; conc, concentration.
§ Approximate dosage calculated from measured food intake.
‖ Rats of the age used in this study normally eat less as they grow older, thus explaining the relatively wide range of dosage received at the three lower dietary levels, which the rats tolerated.

Table 2.—*The Acute Toxicity of Abate in Adult Rats and Mice*

Species	Sex	Route	No.	Batch	LD_{50} (mg/kg)	95% Confidence Limits (mg/kg)	Lowest Dosage (mg/kg) Tested	Lethal	Survival Time* (days) Range	Mean
Rat	M	Oral	58	A	8,600	7,166-10,320	250	6,000	1-13	6
Rat	F	Oral	50	A	13,000	11,304-16,250	375	8,000	1-18	7
Rat	F	Oral	20	B	4,000†	...	2,000	4,000	3-11	6
Rat	F	Oral	20	C	4,000†	...	2,000	4,000	3-4	4
Mouse	M	Oral	50	A	4,700	4,123-5,358	3,000	3,000	1-7	3
Rat	M	Dermal	20	A	>4,000	...	3,000	4,000	...	...
Rat	F	Dermal	20	A	>4,000	...	3,000	>4,000	...	...

* Survival time of animals that died. Groups were observed 22 days or more.
† Approximate value.

caused no deaths among the females. All the affected animals showed the classical symptoms of illness and manner of death associated with organic phosphorus poisoning.

Subacute Oral Toxicity to White Rats.—Cholinesterase values remained normal for the rats receiving 1 mg/kg/day throughout the 44 days of the experiment.

The rats receiving 10 mg/kg/day showed 31% inhibition of RBC cholinesterase after 14 days and 47% inhibition after 44 days. These animals never showed symptoms of organic phosphorus poisoning.

The rats on 100 mg/kg/day developed typical symptoms of organic phosphorus poisoning after three doses when their RBC cholinesterase was inhibited by 64%. Gradual recovery from symptoms occurred while dosing progressed, even though the RBC cholinesterase continued to fall to 87% inhibition after 11 days of dosing. At this point some of the rats were allowed to recover for 32 days without receiving Abate and RBC cholinesterase had returned to a level of 27% inhibition at the end of this time.

The ten rats fed Abate at a dietary level of 2,000 ppm all developed signs of organic phosphorus poisoning, and all showed 100% inhibition of RBC cholinesterase after four days. Eight of the group died between the fifth through the tenth days, but the remaining two survived the 99 days of dosage in spite of continued 100% inhibition of RBC cholinesterase and 80% inhibition of plasma cholinesterase.

None of the animals receiving less than 2,000 ppm developed any symptoms of poisoning. Those on diets containing 200 ppm Abate experienced a progressive inhibition of RBC cholinesterase which was almost complete after two weeks on the diet but then improved gradually to a level of 71% inhibition at the end of the 99 days. No significant cholinesterase inhibition occurred in the groups receiving 20 or 2 ppm of Abate in their diets (mean dosages of 1.8 and 0.18 mg/kg/day, respectively). As might be expected, Abate was slightly less toxic when given in the diet than when given daily by stomach tube.

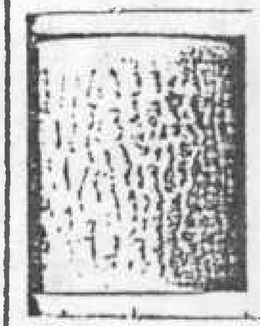
Findings of gross and microscopic pathology of rats from all the above groups were unremarkable.

Additional studies were made in rats to detect possible differences among the three batches. The results (Table 3) confirmed the relevant ones already stated for batch A but failed to reveal any significant difference among the batches.

Oral Toxicity to Rabbits.—No significant inhibition of cholinesterase occurred in the groups receiving 0.1 or 1 mg/kg/day of Abate throughout the 35 days of the experiment.

After seven days of dosage, the rabbits on 10 mg/kg/day had developed 26% inhibition of RBC cholinesterase, and inhibition increased to 47 % at the end of 35 days of dosage. None of these animals developed symptoms of organic phosphorus poisoning.

Eight rabbits were placed on the dosage of 100 mg/kg/day for five days. Three of these animals died, apparently of Abate poisoning. Of the seven rabbits in this group that were autopsied, two showed focal necrosis and two showed diffuse necrosis of the liver. Liver necrosis was not observed in the autopsies of six control rabbits. Neither liver necrosis nor any other pathological evidence of poisoning was present in rabbits on dosages of 10, 1, and 0.1 mg/kg/day for 35 days. The liver injury may or may not be



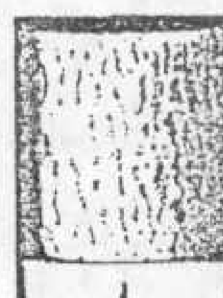


Table 3.—*Effect of Different Oral Dosages of Abate on Several Species**

Species	Batch	Dosages Producing no Illness (mg/kg/day) × days treated	Batch	Dosages Producing Illness or Pathology (mg/kg/day) × days treated
Rat	A	0.12 × 99‡ (12/12, no inhibition of ChE†)	A	100 × 11 (7/7, severe inhibition of ChE; initial illness followed by recovery during dosage)
	A	0.12 × 99 (10/10, no characteristic pathology)		
	A	1.2 × 99‡ (12/12, no inhibition of ChE)	A	150 × 99 (8/10 died in 5-10 days but showed no characteristic pathology)
	A	1.2 × 99 (10/10, no characteristic pathology)	A	150 × 99 (0/11 died, but 11/11 with symptoms and severe ChE inhibition)
	A	1 × 28 (8/8, no inhibition of ChE)		
	B	1 × 28 (8/8, no inhibition of ChE)	A	500 × 4 (4/4 died)
	C	1 × 28 (8/8, no inhibition of ChE)		
	A	1 × 44 (7/7, no inhibition of ChE)		
	A	10 × 44 (7/7, moderate inhibition of ChE)		
	A	10 × 28 (8/8, moderate inhibition of ChE)		
	B	10 × 28 (8/8, moderate inhibition of ChE)		
	C	10 × 28 (8/8, moderate inhibition of ChE)		
	A	11 × 99‡ (14/14, marked inhibition of ChE)		
Rabbit	A	0.1 × 35 (4/4, no inhibition of ChE and no pathological changes in the liver)	A	10 × 30 (1/3, mild changes in liver pathology)
			B	10 × 30 (1/4, mild changes in liver pathology)
	A	1 × 35 (4/4, no inhibition of ChE and no pathological changes in the liver)	C	10 × 30 (2/3, mild changes in liver pathology)
			A	100 × 5 (4/12 died; 6/12 showed focal and 2/12 showed diffuse necrosis of liver)
	A	10 × 35 (4/4, moderate inhibition of ChE and no pathological changes in the liver)	B	100 × 5 (1/4 died; 1/4 showed focal and 1/4 showed diffuse necrosis of liver)
			C	100 × 5 (1/4 died; 1/4 showed diffuse necrosis of liver)
Guinea pig	A	100 × 5 (5/5, normal)		
Dog	Conc§	0.6 × 129 (2/2, normal ChE)		
	Conc§	3.0 × 129 (2/2, marked inhibition of ChE)		
Chicken	A	7.4 × 109 (2/2, normal)	A	15.3 × 109 (2/2, leg weakness after 30 days dosage)
	A	125 × 1 (10/10, normal)	B	125 × 1 (3/10, very mild, brief leg weakness)
			C	125 × 1 (3/10, very mild, brief leg weakness)
			A	250 × 1 (2/6, leg weakness)
			B	250 × 1 (1/2, leg weakness)
			C	250 × 1 (2/2, leg weakness)
			A	500 × 1 (11/11, leg weakness)
			B	500 × 1 (2/2, leg weakness)
			C	500 × 1 (2/2, leg weakness)
			A	1,000 × 1 (5/10 died; leg weakness in all survivors)
Chick duckling	A	10 × 5 (6/6, normal)	A	25 × 5 (2/6 died)
			A	10 × 5 (4/5, mild illness; recovery during dosage)
			A	25 × 5 (5/8 died)

* The number of animals showing the condition specified is expressed as a fraction of the number tested except that in some instances not all members of a group were tested for cholinesterase activity. Each denominator represents a group; no group is listed twice.
† ChE indicates Cholinesterase activity; conc, concentration.
‡ This was the dosage received at 99 days. Food consumption and, therefore, dosage were higher at the onset of feeding when the animals were younger.
§ The material given to dogs was 43% emulsifiable spray concentrate.

accompanied by necrotic lesions in the gallbladder wall or the gastrointestinal tract.

Additional studies were carried out to detect possible differences among the three batches of Abate. When five doses were given by stomach tube at the rate of 100 mg/kg/day, the results (Table 3) were similar to those already described for batch A, and there was no significant difference in the character or degree of pathological changes produced by the different batches. Contrary to the finding in earlier tests, mild pathological changes were found in the livers of some animals that received 10 mg/kg/day, including those that received batch A.

Oral Toxicity to Guinea Pigs.—None of a group of male guinea pigs given a daily dose of 100 mg/kg of Abate by stomach tube for five days showed any sign of organic phosphorus poisoning. The guinea pigs were killed at the end of five days, and autopsy findings were unremarkable. Cholinesterase levels were not investigated.

Oral Toxicity to Dogs.—None of the dogs showed any sign of poisoning. There was no effect of Abate on the plasma cholinesterase of any of the dogs nor on the RBC cholinesterase of either of the dogs given 10 ppm Abate. The RBC cholinesterase of the male dog given 50-ppm Abate fell to 67% of normal during the first week and to 22% of normal in 129 days. The RBC cholinesterase of the female given 50 ppm Abate remained normal through the 60th day, but fell to 50% of normal by the 90th day and remained at that level through the 129th day.

Production of Leg Weakness in Chickens.—The ten hens given single subcutaneous doses of Abate at the rate of 1,000 mg/kg developed leg weakness the day of dosage, and five of the ten died. All survivors had leg weakness, which persisted from 6 to 38 days with a mean persistence of 26 days. All had recovered by 38 days after dosage. The hens receiving 500 mg/kg all developed leg weakness the day of dosage, and all survived. Leg weakness persisted from 11 to 31 days with a mean persistence of 15 days. All these hens had recovered by 31 days after dosage. The response of the chickens to Abate at these dosages were similar to that seen for malathion[4] in that onset of leg weakness was immediate and recovery occurred with time.

All hens given Abate at the dietary concentration of 2,000 and 1,000 ppm died in 30 to 43 days. The hens fed 500 ppm (mean dosage of 15.3 mg/kg/day) developed leg weakness after 30 days of dosage. The hens given 250 ppm (mean dosage of 7.4 mg/kg/day) did not show symptoms of leg weakness at any time during the 108 days of dosage.

Additional acute tests were done to detect possible differences among the three batches of Abate. The results (Table 3) indicate no significant difference among the batches. The lowest dosage tested (125 mg/kg) produced a very mild effect that disappeared within only six days.

Oral Toxicity to Chicks and Ducklings.—All the chicks and ducklings receiving Abate by stomach tube at the rate of 50 mg/kg/day died. Two of six chicks and five of eight ducklings on 25 mg/kg/day died, while the remainder on this dosage all showed signs of organic phosphorus poisoning. The sick birds were generally weak, but typical leg weakness was not observed.

The chicks on 10 mg/kg/day showed no ill effects of any kind, while the ducklings on the same dosage developed minimal signs of poisoning over the first two days but recovered and appeared normal by the end of the five days of dosage.

Autopsy findings for chicks and ducklings were unremarkable. Cholinesterase levels were not investigated in the chicks and ducklings.

Acute Oral Toxicity to Rats of Combinations of Abate and Malathion.—All of the rats given dosage levels of 1/8 LD_{50} Abate plus 1/8 LD_{50} malathion or greater died. Those receiving 1/16 LD_{50} Abate plus 1/16 LD_{50} malathion showed no clinical effect. The results indicate approximately a fourfold potentiation of toxicity where the two compounds are given together at levels approaching the LD_{50} values.

Effect on Reproduction in Rats.—There was no significant difference in number of litters produced (15 from 15 matings), litter size (average, 10.5), viability of young, or incidence of congenital defects between rats receiving Abate at a dietary level of 500 ppm and normal members of the rat colony, even though the dosage caused signs of poisoning in some rats.

The RBC cholinesterase of the mothers was inhibited by 90% after 48 days of dosage, while that of their healthy 21-day-old young was inhibited by 30%.

Summary

In laboratory animals Abate produces signs, symptoms, and type of death typical of those associated with other organic phosphorus compounds. In large enough doses Abate will inhibit cholinesterase, and it does this more promptly and to a greater extent for RBC cholinesterase than for plasma cholinesterase.

The acute oral lethal dose (LD_{50}) for Abate in rats and male mice is 4,000 mg/kg or greater. Restrained female rats tolerate a dermal dose of Abate of 4,000 mg/kg without clinical effect, but the same dosage killed two of ten males.

Rats, rabbits, guinea pigs, and chickens tolerate a daily oral dosage of Abate of 10 mg/kg without observable clinical effect, and dogs tolerate a daily dosage of 3 to 4 mg/kg, the highest rate at which they were dosed. Rats and rabbits tolerate at a dosage of 1 mg/kg/day for extended periods of time without detectable effect on cholinesterase.

Focal or diffuse necrosis of the liver was present in some rabbits given Abate at the rate of 100 mg/kg/day for five days, and mild pathological changes occurred in some that received 10 mg/kg/day for 30 days. No liver necrosis or other characteristic pathology of the liver was present in rabbits given 1 and 0.1 mg/kg/day for 35 days, and no relevant pathological effects of any kind were found in the other species studied.

Abate in a dosage of 500 mg/kg or higher produced a rapid onset of leg weakness in chickens from which the survivors recovered within 36 days or less. A single dose at the rate of 125 mg/kg produced a very mild leg weakness in a few chickens, but they recovered promptly. When Abate was fed to hens daily, those receiving an average dosage of 15.3 mg/kg/day developed leg weakness after 30 days, but hens that received an average dosage of 7.4 mg/kg/day did not show symptoms of leg weakness at any time, although they were fed Abate for 108 days. Thus, the effect of Abate on chickens is similar to that of malathion.

Abate and malathion show an approximately fourfold potentiation of toxicity when the two are given together at levels approaching the LD_{50} values.

Male and female rats maintained on a dietary intake of Abate large enough to produce marked inhibition of cholinesterase and some symptoms of poisoning reproduced normally with no decrease of number of litters, litter size, or viability of young and with no increase of congenital defects.

Batches of technical Abate differ somewhat in their acute oral LD_{50} values, but the toxicity of all tested was low. More important, there were no significant differences in the effects of different batches in regard to their ability to produce pathological changes in the liver in rabbits, leg weakness in chickens, or cholinesterase inhibition in rats following repeated dosage.

Richard Moore and Ralph Linder gave technical assistance with the animals; Miss Florence Whitfield performed the cholinesterase determinations, and Mrs. Martha Bey assisted in the preparation of tissue for microscopic study.

The technical Abate and the emulsifiable concentrate of the compound used in this study was supplied without charge by the American Cyanamid Laboratories, Princeton, NJ.

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United States Department of the Interior

FISH AND WILDLIFE SERVICE
PATUXENT WILDLIFE RESEARCH CENTER
LAUREL, MARYLAND 20811

August 7, 1980

Dr. Stanley R. Joseph
Chief, Mosquito Control
Maryland Department of Agriculture
Parole Plaza Office Building
Annapolis, MD 21401

Dear Doctor Joseph:

Your inquiry about the effects of Abate on muskrats was followed by a letter from the Dorchester County Commissioners. In response, we have done several things.

The first was to communicate with our Service's wildlife disease laboratory in Madison, Wisconsin. The head of that laboratory, Dr. Milton Friend, and a veterinary diagnostician, Dr. Louis Locke, were extremely interested in this problem. They are not only willing, but anxious, to examine muskrats from areas where the problem occurs. Dr. Locke has called Mr. Lesser about getting material for examination. If there are other individuals whom he should call about getting affected muskrats, please let us know. We believe that this approach is extremely important, for muskrats do suffer from serious diseases. Dr. Friend and his staff are also well aware of chemical problems and will be on watch for them.

The second line of approach will be to analyze muskrats and muskrat foods from a Dorchester County marsh soon after an Abate application, preferably after one of the later applications of the season. We have an excellent analytical laboratory and we are willing to analyze several samples of both foods and muskrats. We are arranging with American Cyanamid Co. to receive analytical standards for Abate and its chemical metabolites and also a copy of the latest analytical methods. It looks as if the company will wish to analyze samples in parallel with our laboratory for comparative purposes.

As to the collecting of these samples, we believe that it would be best for them to be collected by Mr. Lesser working jointly with a recognized representative of the trappers. This joint effort might avoid questions about how the samples were collected and how representative they were. Techniques for collecting, storing and shipping samples will be supplied later.

TELEPHONE—AREA CODE 301 776-4880 (MARYLAND EXCHANGE)
TELEGRAMS—FISH AND WILDLIFE SERVICE, WASHINGTON, D.C. 20240

Our next approach was to review the [illegible] effects. Our biologists found no mention of tests with muskrats, but found that quite a bit of work has been done with rats and other mammals. Rats and muskrats are rather closely related rodents and it is likely that they would react to Abate in similar ways. Work with other mammals, and even birds, is also informative. We will review some of these studies in this letter in an effort to put the muskrat exposure into perspective.

As you know, the big question in all toxicity problems is "How much?" All chemicals are toxic at some high level and all are nontoxic at some low level. As little as 2 ounces of table salt can kill a man, yet small amounts are necessary for health. With pesticides, animals survive and breed well with low exposures, but can be harmed by heavy doses. So we must ask whether muskrats are exposed to amounts of Abate that should harm them in light of work with other animals.

Abate is famous for killing mosquito larvae at low concentrations. In Dorchester County, Lesser's men apply 1.5 ounces per acre of fluid Abate, or about 0.048 pounds of active ingredient, per acre. This would yield 0.035 ppm (parts per million) in water six inches deep. Even allowing for uneven distribution, which is inevitable with aerial applications, very little material is applied. Some areas are treated once or twice a season and the maximum is five times.

Exposures are brief, for no study has shown Abate to last long in the environment. One study found no more than 0.3 ppm of Abate in algae and detritus after applications. Even after unusually heavy applications, only trace amounts were found in water or mud, and no residues at all were found in some tests. Thus Abate is extremely different from the persistent chemicals such as DDT.

In all, we see no reason to suspect that muskrats could be exposed to much more than a fraction of a part per million in food or water, and we see no reason to suspect that an exposure would be lasting.

In our own work, we found that it takes susbstantial amounts of Abate to kill baby birds. When Abate was mixed into the diet and fed for five days, half of the young birds were killed only when Abate was present at 92 ppm for bobwhites, 162 ppm for pheasants, 260 ppm for Japanese quail, and 894 ppm for mallard ducks. We see no way in which birds could get anything approaching such exposures from Abate applications. Even a tenfold error of application would not do it.

When applied to the skin of rabbits, Abate was not very dangerous. It took 1930 mg/kg (milligrams of chemical per kilogram of body weight) to kill half of the males and 970 mg/kg to kill half of the females. For comparison, TEPP, another organophosphate insecticide, killed at 5 mg/kg. Dieldrin, a dangerous organochlorine insecticide, killed at 45 mg/kg. Muskrats probably would have no more than trace amounts of Abate reaching the skin for short periods.

In eye exposure, there was no effect when drops of Abate (0.1 ml) were placed in the eye of rabbits. Abate proved exceptionally mild in this test.

In long-term studies in which Abate was added to the daily diet of rats, 350 ppm allowed rats to survive the 90-day test without any microscopic tissue damage. This high dosage did, however, markedly inhibit the enzyme cholinesterase. This enzyme is usually measured as an index to the severity of exposure to organophosphates, of which Abate is one. The amount of inhibition dropped at lower dosages. No worrisome level of inhibition appeared in rats receiving 2 ppm. This inhibition is reversible, and low levels of inhibition are not considered dangerous to behavior of reproduction.

The U.S. Public Health Service did much work with Abate in various species, including man. They found that it took thousands of mg/kg to kill rats or mice in single doses (the LD_{50}). Such amounts would be obtained only in dosage studies, never in the wild.

Rabbits given 10 mg/kg every day for 35 days had no tissue damage.

Rats given large amounts of Abate in their feed (500 ppm), enough to produce symptoms of poisoning, still reproduced well. This suggests that Abate should not affect muskrat reproduction, for the data at hand indicate that muskrats would be exposed to only fractions of a ppm of Abate, and then only for brief periods.

The Public Health Service concluded, "Rats, rabbits, guinea pigs, chickens, and ducklings will all tolerate a daily oral dosage of Abate of 10 mg/kg without observable clinical effects, and rats and rabbits tolerate Abate at a dosage of 1 mg/kg/day for extended periods without detectable effect on cholinesterase." In other words, the no-effect level was above 1 mg/kg/day. A muskrat should be able to eat a small, but visible, amount of Abate every day with no sign of effects.

The Public Health Service then tested Abate in human volunteers, for they hoped to be able to use it for mosquito control in tanks of drinking water in the tropics. Men took 1 mg/kg/day for a month. This amounted to the easily visible amount of 64 mg per man per day. There was no effect in man. Abate consequently is being used in tanks of people's drinking water to this day. If the amount of Abate that will control mosquitoes can be ingested by man on a continuing basis, it does not seem likely that intermittent and short-lived exposures in the marsh could harm muskrats.

A study with sheep revealed no effect from such heavy doses as 84 mg per sheep given daily for 422 days, or 5 mg/kg/day given for 157 or 187 days, or 20 ppm in drinking water for 176 or 184 days. Even the lambs born to treated parents did well.

A study with cattle did reveal effects on parents and young, but very few animals were tested, and each was given nearly half a gram of Abate every day.

March 3, 1978 iii Conclusions

To us, one of the most revealing things about Abate is that it kills so few invertebrates other than mosquitoes. Mosquitoes and midges are controlled with applications that do no visible harm to dragonfly larvae, phantom larvae (Chaoborus) or crustaceans such as copepods and ostracods. Such organisms as these are highly sensitive to most insecticides. There is strong reason to believe that when the small crustaceans are safe, all of the higher animals are safe. The small crustaceans are not only highly sensitive to chemicals that kill insects, but they are soaked in the solution just as the mosquitoes are. One expects to see the crustaceans going out long before higher animals are affected. The same thing is true of fish, yet we have heard no complaint of fish being killed in the marshes by Abate.

In view of all this information from sources that have no reason to defend the product, we consider it unlikely that Abate is the cause of the muskrat problem. However, we hope to run analyses to determine the exposure of muskrats to Abate, for surprises do occur in this work and we must not fail to investigate such possibilities. It seems most probable, however, that disease investigations will turn up the answer to the muskrat problem. These investigations will be made gladly by our Madison laboratory.

Sincerely yours,

Lucille F. Stickel

Lucille F. Stickel
Director

Enclosure

cc: Dorchester County Commissioners
Mr. Cyrus Lesser
Mr. Lombardi, Abate Prod. Mgr.
Dr. Lublinkhoff, Head R&D for Abate et al.

MARYLAND

DEPARTMENT OF AGRICULTURE

Harry Hughes, Governor
Wayne A. Cawley, Jr., Secretary
XXXXXXXXXX, Deputy Secretary
Hugh E. Binks,

Parole Plaza Office Building, Annapolis, Maryland 21401
Phone 301—269-2161

OFFICE OF PLANT INDUSTRIES AND PEST MANAGEMENT
MOSQUITO CONTROL SECTION

July 1, 1982

Dr. Sarah J. Taylor, Director
Coastal Resources Division
Department of Natural Resources
Tawes State Office Building
Annapolis, Maryland 21401

Dear Dr. Taylor:

The Memorandum of Understanding on the Coastal Zone Management Program between the Department of Agriculture and the Department of Natural Resources requires that the Mosquito Control Section provide a statement on the impact that a jointly funded study has had on mosquito control activities in this State. This letter is intended to meet the requirement of the Memorandum of Understanding.

The study was designed to evaluate the impact of three water management techniques on Chesapeake Bay high salt marshes. Data collected since 1978 provided detailed information on marsh ecology as well as the impact of water management on mosquito populations. The study demonstrated the limitations of water management systems with no outlets to tidal water (closed systems). Although these closed systems controlled salt marsh mosquitoes in pothole conditions on clay marshes with a shallow peat layer, mosquito control was unsatisfactory when larvae developed in shallow sheet water. Closed systems failed to achieve mosquito control in marshes with a deep peat layer because fish, the principal biological control organisms, could not survive summer droughts and failed to penetrate shallow standing water. Measurements showed that dissolved oxygen was a limiting factor for fish survival. The management objective of closed systems, i.e., to eliminate the need to use pesticides, was not achieved as several of the sites required repeated sprayingfor larval mosquito control during the 1980 and 1981 seasons.

Water management systems with sill outlet ditches were found to be the best compromise to achieve mosquito control, promote and preserve predatory fish populations, and limit the invasion of undesirable vegetation on the marsh. Systems are now being constructed with sill outlets.

Dr. Sarah J. Taylor July 1, 1982

Although open systems (ditches of full depth connected to tidal water) effectively reduced mosquitoes, they also favored greater vegetation changes and the invasion of undesirable shrubs.

The Coastal Zone Administration of the Department of Natural Resources contributed the major portion of funding. MDA supported the study and subcontracted a significant part of the study to the Smithsonian Institution. The results of the study were used immediately to design workable systems for water management. Reports by Dr. Dennis Whigham and Cyrus Lesser have assisted the Maryland Mosquito Control Advisory Committee in the preparation of "Standards for Maryland Open Marsh Water Management" which are now in use.

A copy of these Standards is attached.

Sincerely,

Stanley R. Joseph

Stanley R. Joseph, Chief
Mosquito Control Section

SRJ:sd

Enc.

STANDARDS FOR MARYLAND OPEN MARSH WATER MANAGEMENT (MOMWM)

The need and demand for improved control of salt marsh mosquitoes; primarily Aedes sollicitans; exists in many parts of Maryland, particularly the southern Eastern Shore region. It is accepted that the fundamental requisite for the control of Ae. sollicitans is the control of the larvae by either chemical means (larviciding) or physical means (water management). Whereas chemical control poses several problems; including environmental contamination, high cost, temporary results, and eventual resistance by the mosquitoes; it is concluded that control by water management is the preferred control technique.

The basic principle of water management mosquito control techniques is to facilitate access of larvivorous fish to the mosquito breeding areas and/or cause removal of water from the breeding areas before the mosquito larvae can complete their development.

The Maryland Mosquito Control Advisory Committee has investigated various strategies of water management for use in the State since 1976. In order to identify the management technique(s) most suited for use in Maryland, certain standards are necessary. These standards were developed through practical experience and comprehensive ecological studies and shall be used as a guide in future water management projects and be incorporated in all permits issued for mosquito control marsh management projects. These standards will be periodically reviewed and revised if necessary.

I. Objective

A. The primary objective is to provide a management technique that will control the larval production of all species of salt marsh breeding mosquitoes: Aedes sollicitans, Aedes taeniorhynchus, Aedes cantator, Anopheles bradleyi and Culex salinarius.

B. Reduction in the use of insecticides - After the completion of a water management project, mosquito control will be achieved, therefore the use of larvicides on that area will be eliminated.

C. The application of MOMWM shall, to the extent possible, minimize the negative impact on floral and faunal composition of the salt marsh/estuarine ecosystem.

D. The technique must be cost effective - Inasmuch as public funds will pay for this management it is essential that these funds be used to provide the intended results at a reasonable cost.

II. Need: The use of MOMWM will be based entirely on the need for mosquito control as determined by larval inspections.

III. Implementation

A. Because the ecological requirements necessary for the breeding of all genera of salt mosquitoes are reflected in the vegetational character of the marsh, this character can be used to determine potential breeding marshes. In Maryland, the plant species associated with high marsh, i.e. infrequently flooded by rains, spring or storm tides; therefore indicative of mosquito breeding habitat are: Distichlis spicata; Spartina patens; short form Spartina alterniflora; small areas of Juncus Roemerianus, Scirpus Olneyi and Typha spp. in association with the previous three species; Phragmites communis, Scirpus robustus, and (in some instances) Panicum spp. Water management will not be employed on marshes subject to regular floodings (greater than 8 days per month) or daily tides. Non-breeding marshes are vegetationally characterized by tall form Spartina alterniflora; Zizania aquatica; extensive stands of either Typha spp., Scirpus Olneyi or Juncus Roemerianus; and similar species of vegetation. Permanent ponds on the salt marsh do not provide breeding sites for mosquitoes and will not be drained.

B. All alterations must directly affect mosquito breeding sites.

C. An experienced mosquito control entomologist, wetland biologist, or both shall stake out all of the alterations to be constructed. The amount of construction done will be the minimum required to satisfy the objectives of MOMWM.

IV. Alterations: Four types of alterations (tidal ditches, semi-tidal ditches, ponds and pond radials) will be used. To a degree, the type of alteration used will be dependent on the type of marsh being managed. Darmody and Foss (1978) define three types of marsh in Maryland: Coastal, Submerged Upland and Estuarine.

Coastal type marshes have a higher salt content in the soil than the other marsh types and are characterized by vast swards of S. alterniflora and S. patens. Coastal marshes occur along the margins of Chincoteague and Assawoman Bays in Worcester County. It is the dominant marsh type in Worcester County and constitutes all of that county's mosquito breeding salt marsh.

Submerged upland type marshes have developed over areas which were formerly uplands and are being submerged by the slowly rising sea level. These marshes are characterized by relatively thin organic soils overlaying older mineral soils developed from wind-blow silts or sands. The dominant vegetation consists of J. Roemerianus, S. patens, D. spicata, S. alterniflora, S. Olneyi and P. communis. It is the dominant marsh type in Maryland, and is found primarily in Dorchester and Somerset Counties where it is the predominant mosquito breeding marsh type.

The estuarine type marsh occurs in all counties along Chesapeake Bay and the Atlantic Coast. This marsh type is found primarily along streams and rivers which drain into Chesapeake Bay. The marshes develop from the silting in of streams, estuaries or bays. They may also develop from the accumulation of sediments in tidal streams as estuarine meanders. The dominant vegetation in the brackish and saline areas of this marsh type is S. alterniflora, S. patens, D. spicata, Spartina cynosuriodes, S. Olneyi and J. Roemerianus. This is the dominant marsh type for salt marsh mosquito breeding in the Western Shore region and is also common along Pocomoke Sound in Somerset County and Fishing Bay in Dorchester County.

A. Tidal Ditches

1. Tidal ditches are the most effective alteration to eliminate mosquito breeding and are the preferred type of ditch to be used on coastal marshes and on some estuarine marshes. On submerged upland marshes the use of tidal ditches will be restricted to the upland edge as a "band ditch" and when existing upland drainage ditches outlet to the marsh that part of the ditch traversing the marsh to a tidal drain may be cleaned so as to assure tidal flow.*

2. All tidal ditches will be dug with suitable equipment, preferably with a rotary ditcher. When a rotary ditcher is not available or cannot be used other equipment types, such as amphibious cranes or backhoes, are acceptable provided that spoil taken from the ditches is graded to as near marsh level as possible. Spoil dug with a crane or backhoe should be placed on opposite sides of the ditch so as not to form a continuous line of spoil which would impede water movement across the marsh surface.

3. Tidal ditches should be dug to a depth of two to three feet, with the deeper ditches being preferred.

4. Main tidal ditches are used to provide tidal circulation through large areas. The ditches can be connected to a tidal source at one or more points. The location of the main ditch is determined by the distribution of mosquito breeding sites and the proximity of a tidal source.

5. Lateral tidal ditches connect breeding sites to main ditches, natural tidal creeks or other laterals. Such laterals often dead end at a breeding site.

6. All previously constructed mosquito or other ditches that are breeding sites will be cleaned.

7. Wherever possible, spoil taken from a tidal ditch will be used to fill nearby mosquito breeding depressions.

*underlined sections will be discussed by the membership of the Advisory Committee.

B. Semi-tidal Ditches

1. Semi-tidal ditches are the preferred type of ditch to be used on submerged upland marsh that can be classified as open marsh, i.e. not bordering the upland edge and not under the impounding influence of old dikes or roads. Semi-tidal ditches may also be used on estuarine marshes.

2. Semi-tidal ditches will be constructed according to the specifications given for tidal ditches except that the outlet of main ditches will contain a sill which will not allow complete drainage. This sill will be approximately 100 feet long and 6 to 10 inches below the marsh surface (see Fig. 1).

3. The semi-tidal ditches will allow drainage of excess surface water, thus eliminating sheet water breeding sites, and flood frequently enough by spring or storm tides to maintain a water quality sufficient for fish survival. If these results are not achieved, the depth of the sill shall be lowered.

C. Ponds

1. Where numerous mosquito breeding depressions are concentrated in a limited area, a pond alteration will be utilized.

2. Pond construction is accomplished by a rotary ditcher, amphibious crane, backhoe or other suitable equipment.

3. Ponds should be 18 inches or 24 inches in depth to promote waterfowl and wading bird use and the growth of submerged aquatic vegetation.

4. To ensure fish survival in the ponds during droughts a resevoir ditch of at least three feet depth shall be constructed along at least two sides of the pond edge.

5. Pond shape may be either linear or take the shape of the breeding area.

6. Islands shall be left in the pond when possible to provide additional edge cover within the pond.

7. Pond spoil should be graded as low as possible without undue disturbance to the nearby non-breeding marsh surface. Pond spoil shall be used to fill mosquito breeding depressions when possible.

D. Pond Radial Ditches

1. Mosquito breeding sites located near a permanent natural or constructed pond shall be connected to the pond by pond radial ditches. These radial ditches will provide access for fish to devour mosquito larvae at the breeding sites.

2. All pond radials shall be constructed with the type of equipment previously mentioned for tidal and semi-tidal ditches.

3. Spoil from the radial ditches shall be disposed of in a similar manner as described for tidal and semi-tidal ditches.

4. To prevent drainage of a natural permanent or constructed pond by muskrats, or other natural factors, all pond radials shall end no closer than 50 feet from a tidal ditch or creek.

V. Other Techniques: Impoundments, closed ditch systems and other types of management not described here are not MOMWM.

REFERENCE CITED

Darmody, R.G. and J.E. Foss. 1978. Tidal Marsh Soils of Maryland. Maryland Agr. Exp. Sta. Univ. of Maryland, College Park, Maryland. 69 pp.

JAMES B. COULTER
SECRETARY

LOUIS N. PHIPPS, JR.
DEPUTY SECRETARY

STATE OF MARYLAND
DEPARTMENT OF NATURAL RESOURCES
TIDEWATER ADMINISTRATION
TAWES STATE OFFICE BUILDING
ANNAPOLIS 21401

(301) 269-2784

August 10, 1982

Mr. Robert H. Forste
Asst. Science Advisor
Research Division - State Dept. of
Legislative Reference
Room 110
90 State Circle
Annapolis, Maryland 21401

Dear Mr. Forste:

Since our phone conversation two weeks ago, I have had printed 20 copies of one part of the Open Marsh Water Management Study by Dr. Cyrus Lesser as well as 20 copies of a letter with operational Standards for Maryland Open Marsh Water Management. Enclosed are 15 copies of each of these items which I thought would be useful for you as well as the Governor's Task Force which you are coordinating for mosquito control.

Also, since our conversation two other events have happened which might be helpful to you. First, I have received the final report from Dr. Dennis Whigham, representing the other half of the Open Marsh Water Management Study that was funded. I will have 20 copies made of this as well and forward them to you in 2 weeks.

Second, I met with a Dr. Milan Trpis, Professor of Medical Entomology in the Department of Immunology and Infectious Diseases, School of Hygiene and Public Health, Johns Hopkins University. In my discussions with him, I found out that he was doing research on bascillae, which when ingested by mosquito larvae are lethal to them but not to other aquatic or bird life. I explained to him what you were to be doing with the Task Force on mosquito control and also told him about the scientific articles you had on the use of biodegradable films for mosquito control. He expressed an interest in these articles, so I told him that I would write and ask you to send them to him. He may also be someone, due to his experience, that you may want to involve in your effort.

TTY FOR DEAF — BALTIMORE 269-2609, WASHINGTON METRO 565-0450

In case you do wish to contact him as well as the other people that have been working with mosquito control techniques; here is a listing of their names, addresses and phone numbers:

Mr. Tom Dolan (Open Marsh Water Management) whom you have already contacted.

Mr. Stan Joseph, Chief (Open Marsh Water Management)
Mosquito Control Section
Office of Plant Industries and
Pest Management
Md. Dept. of Agriculture
Parole Plaza Office Building
Annapolis, Md. 21401 269-2961

Dr. Cyrus R. Lesser, Entomologist (Open Marsh Water Management)
Same Address as above

Dr. Dennis Whigham, Acting, (Open Marsh Water Management)
Assoc. Director for Science
Chesapeake Bay Center for
Environmental Studies
Smithsonian Institution
P. O. Box 28
Edgewater, Md. 21037 798-4424

Dr. Milan Trpis
The Johns Hopkins University
School of Hygiene and Public Health
615 N. Wolfe Street
Baltimore, Maryland 21205 955-3475

If I can be of further help, please let me know.

Sincerely,

Sarah

Sarah J. Taylor, Ph.D.
Director, Coastal Resources Division

SJT/dmt
Enclosures
cc: Joseph Whigham
Cyrus R. Lesser
Millan Trpis
Tom Dolan
Stan Joseph

FINAL REPORT

INTEGRATED MOSQUITO CONTROL IN MARYLAND WETLANDS AND ENVIRONMENTAL EFFECTS

Grant No. 58-7B30-8-12

Stanley R. Joseph
Chief, Mosquito Control
Maryland Department of Agriculture

The integrated pest management program for mosquito control continued in Maryland with some modifications and improvements. The primary reasons for mosquito control in Maryland are to reduce annoyance and prevent the transmission of encephalitis and dog heartworm. Improvements and changes during the period of the Grant include the following:

Coordination - The Maryland Mosquito Control Advisory Committee continued to coordinate mosquito control in the State. This committee has representation from the Maryland Department of Agriculture; U.S. Department of the Interior, Fish and Wildlife Service; U.S. Environmental Protection Agency; U.S. Corps of Engineers; National Marine Fisheries Service; Maryland Department of Natural Resources and the University of Maryland.

The Advisory Committee continued to meet two to three times each year. Meetings were held to discuss the status of the program and field inspections were made to review the status of work on the wetlands. The committee has developed and approved a Standard Operating Procedure for Open Marsh Water Management work in Maryland that will expedite the permit system.

Training - Seven members of the mosquito control staff attended the U.S. Public Health Service, Center for Disease Control course, "Control of Mosquito-borne Diseases". This course improved the staff's knowledge of the principles of integrated mosquito control.

In addition, MDA staff also attended and participated in the annual meetings of the American Mosquito Control Association, Northeast

Mosquito Control Association, Mid-Atlantic Mosquito Control Association, New Jersey Mosquito Control Association and a 3-day meeting, "Water Management Workshop for Mosquito Control" sponsored by the New Jersey Agricultural Experiment Station. Many public meetings were attended by MDA professional staff to discuss mosquito control and to educate the community as to what individuals can do to reduce mosquito breeding. A pamphlet containing information on mosquito biology and control was prepared, printed and distributed to the public.

Surveillance - A monitoring program for larvae and adult mosquitoes has been developed to determine the need to initiate control measures and to evaluate the effectiveness of the control work. A series of salt marshes known to be consistent breeding areas has been marked as indicator sites. These areas are checked weekly during the breeding season, more frequestly if there are high tides or heavy rainfall.

Unusual conditions during much of 1980 and 1981 affected the surveillance program and added to our understanding of the factors that influence fish survival on the high salt marshes of Maryland. Summer drought in 1980 and severe winter cold in 1980-81 decimated predatory fish populations so that the 1981 mosquito breeding cycles continued without interruption and expanded into many atypical areas. This extensive and repetitive breeding exhausted resources of the Mosquito Control Section and necessitated emergency and deficit funding by the State. During the period, *Aedes sollicitans* breeding was noted in such atypical areas as large ponds and broad zone *Juncus roemerianus.* The resulting adult mosquitoes emerged in large numbers despite intensive larvicide applications.

Efforts continued to establish action thresholds for larvae and adults. Of the 192,371 acres of tidewater marsh on Maryland's Eastern Shore, 26,000 acres have now been documented as *Aedes sollicitans* breeding sites. This total may eventually reach 30,000 to 35,000 acres. Water management is constantly reducing the total area, but the pace needs to be increased. The action level for larviciding salt marshes is five larvae per dip over 50% of the salt marsh. This is a subjective criteria that may vary with the proximity to populated communities. Breeding is defined as (1) isolated 1-15% of the area; (2) variable 15-50% of the area; (3) widespread over 50%.

Thresholds for adult control activities have been developed for many areas of the State. Generally five adults landing on an observer per minute will elicit citizen complaints. An average of 20 adults landing per minute over a wide area is considered severe enough to schedule aerial adulticiding. However, high costs and short periods of effectiveness emphasize the limited value of aerial adulticiding (six times as costly per acre as ground applications). Larval control and water management are the key measures to success in controlling *Aedes sollicitans.* Adult surveillance was implemented on a countywide basis in several areas of the State during 1980 to provide data for scheduling ULV adulticiding. This change, coupled with a comprehensive larvicide program, resulted in reducing the dispersal of adulticide chemicals by an average of 41% under that applied in the same area in 1979.

Several adult surveillance techniques are being used to determine the need for adulticide applications.

1. Landing rate counts are made daily in some areas at strategic locations. This information is utilized either to schedule spraying or cancel operations if few mosquitoes are present. Another technique of greater practical value is to train sprayer operators to take counts before scheduled applications. If counts are low, entire communities may not need spraying or some sections of large developments may be eliminated from the schedule. This technique requires additional time, but has produced significant cost reductions.
2. New Jersey light traps are also used in the surveillance program. Trap counts sometimes show high mosquito counts in areas where adult activities is limited to night biting species. The limitations of traps has been the time and cost of processing collections promptly.
3. Training community contacts to collect and relay data on mosquito annoyance is also a helpful procedure. This is being developed in several urban counties and appears valuable in supplementing other data.

<u>Chemical Control</u>:

Ground or aerial applications of insecticide (Cythion, Dibrom, Abate, Dursban and Flit MLO) are included under temporary control activities. The volume of insecticides used in the temporary control program since 1977 are shown in Table 1. Although program growth continued through 1981, the use of Cythion for adult control declined by 28% from peak use in 1978. This reduction is directly attributable to sound pest management practices that include use of surveillance data, better timing of applications, and increased emphasis on larval control by chemicals and water management.

Maryland law requires all pesticide applicators to obtain permits in order to apply toxicants to waters of the State. Permits are reviewed by the State Department of Health and Mental Hygiene and the Water Resources Administration, Department of Natural Resources. One concern of these agencies was the possible toxicity of Abate mosquito larvicide to oyster larvae, since oysters constitute an important and very valuable resource of the Chesapeake Bay.

The toxicity of Abate to oyster larvae was evaluated for concentrations ranging from 0.006 ppm to 5.0 ppm under laboratory conditions. It was calculated that in the field, following an aerial application of Abate at the rate of 0.053 Kg/hectare, oyster larvae would be exposed to Abate concentrations ranging from 0.0006 ppm to 0.006 ppm, depending on water depth. In this concentration range, Abate elicited no toxic response from the oyster larvae. Concentrations above 0.20 ppm Abate cause significant mortality in the test population and concentrations above 1.0 ppm caused 100% mortality. However, Abate concentrations at the level inducing oyster larvae mortality would never be encountered in the field when Abate is applied according to label directions. Oyster spat (the smallest of the shelled form of this species) were exposed to Abate concentrations ranging from 0.10 ppm to 10.0 ppm, but no toxicological effect was noted. It was concluded from this laboratory evaluation that the use of Abate according to label recommendations creates no hazard to the important oyster fishery in the Chesapeake Bay region.

Bacillus thuringiensis israelensis (BTI) is a biological agent of recent development that has shown promise in controlling mosquito larvae. Maryland Department of Agriculture staff has evaluated two of the three existing formulations for use against *Aedes sollicitans*. To date these formulations (Vectobac® and Teknar®) have not produced results comparable to Abate at label rates. The third formulation Bactimos is scheduled for evaluation in the near future. In addition to higher cost for the control agent, application rates of 1 to 5 gallons of finished spray required on the label would add tremendously to the cost of aerial applications. For the Maryland program, the material should be applicable by the low volume technique.

Monomolecular films have generated interest in the state. Field testing is planned, a rather complete file on this group of compounds has been accumulated.

Source Reduction - Open marsh water management is the most important phase of MDA's salt marsh mosquito control program. In 1980 a total of 2,643 acres of mosquito breeding salt marsh was subjected to open marsh water management in four counties. This is the most acreage completed in any calendar year in the history of the program. This record was achieved due to improvements in personnel and equipment.

Additional water management work is planned in two more counties. Corps of Engineers permits have been obtained and construction is scheduled for fall of 1982 and spring of 1983.

Research:

Three concurrent studies have assisted MDA in applied research designed to evaluate techniques of water management on Chesapeake Bay wetlands. The studies were based on open marsh water management (OMWM) as developed and used in New Jersey salt marshes. Experience has shown that Maryland marshes responded differently to grid ditching as performed prior to 1975 and were different in several respects from New Jersey marshes. Therefore, the studies were designed to provide definitive answers about the effectiveness and environmental impact of several variations of OMWM as used in New Jersey.

Studies demonstrated that water management can be highly effective in controlling salt marsh breeding mosquitoes. Each of the three management techniques evaluated (tidal, semitidal, and nontidal) provided equal mosquito control on the Davis Island study area of Somerset County. However, the closed system technique was found to be ineffective for mosquito control on secondary study sites where mosquito breeding was found in surface water swales or there was a deep peat soil.

The use of the management systems by marsh surface macro-invertebrates such as Melampus snails, Amphipods and Isopods was measured, but no clear conclusions can be made as to the impact of the management techniques on these organisms. Populations of these animals are relatively low on the Davis Island study area.

The water management systems each provided additional habitat for fish, shrimp, and crabs, and were used by a large and varied population of these animals. Because of this, marsh management practices may enhance production in the estuarine food web. However, on secondary study areas in the Deal Island and Elliott Island areas, fish kills were observed in closed ditch systems. The cause of the fish kills is thought to be low levels of dissolved oxygen. The dissolved oxygen content of the water in the closed ditches was observed to be near zero ppm during early morning and evening. The low dissolved oxygen level is speculated to have been caused by a high biological oxygen demand caused by decay of organic matter in the ditches. The origin of this organic matter was either the peat layer in which the ditches were constructed, large stands of submerged aquatic vegetation, or a combination of both.

The drainage impact of the open tidal ditches caused significantly lower water table elevations on the open plot as compared to the other treatment plots and the control plots. The closed plot displayed a wide range of water table elevations between years, as a function of the amount of rainfall received on the study area. The water control system maintained the most stable water table elevation during the two years of study.

To achieve the desired level of mosquito control on Chesapeake Bay salt marshes, it will be necessary to employ a tidally influenced water management technique. A combination of open and water control ditches will provide effective mosquito control with the minimum degree of alteration to the wetlands, and produce the least negative environmental impact. In conjunction with a system of tidal and semitidal ditches, ponds should be constructed to provide habitat diversity and improve wildlife habitat. These ponds can vary in size, but should not exceed 0.08 hectare.

Large scale closed ditching projects should not be employed in future mosquito control water management projects on Maryland's Chesapeake Bay wetlands because of the observed failure of closed systems to control mosquitoes and negative impacts on fish. Small closed ditching projects such as pond radial ditches, in conjunction with tidal or semitidal ditches are an acceptable and encouraged management technique.

As a result of this study and the study of Lesser and Saveikis (1979), the Maryland Mosquito Control Advisory Committee has been able to develop standards for what constitutes acceptable marsh management techniques for mosquito control in Maryland. These standards are appended (Appendix B) and are very different from the standards for open marsh water management used by New Jersey. Therefore, the techniques used in Maryland should not, in the future, be termed open marsh water management. A more proper term for the water management program in Maryland would be Maryland open marsh water management (MOMWM).

Cost Evaluations:

Some reports have questioned the cost effectiveness of marsh water management work. The following information is presented for Maryland.

Larviciding costs include the per acre contracted cost for the aircraft used (MDA contracts for all aerial application), cost of insecticide and surveillance. The aircraft used for cost comparison is a DC-3 which is used to larvicide large tracts of salt marsh with an Abate 4E/water solution at the rate of 4.0 fluid ounces of solution per acre. Aerial larviciding costs for 1978 through 1980 are presented in Table 2. The average cost per acre per treatment over the three-year period is $1.60.

The cost of OMWM consists of rental fees for assigned heavy equipment units, operator salaries and benefits, and miscellaneous expenses. OMWM costs for 1978 through 1980 are presented in Table 3. The cost per acre of OMWM over the three-year period averaged $126.00. This average is based on various types of equipment ranging from tractor-backhoes to rotary ditchers, and work in different parts of the State.

A ten-year effective life span of a mosquito control ditch is reasonable and an even longer period of effectiveness can be anticipated. Using information provided by Grant and Ireson (1970), we can project the cost of larviciding in the future at fixed rates of inflation. For an inflation factor of 10% for 10 years, the present cost per acre for larviciding is multiplied by a factor of 15.94 (see Table 4) to project the total cost for the 10 year period. For an inflation factor of 10% for 15 years, the present cost of larviciding per acre is multiplied by a factor of 31.77.

As can be seen from Table 5, we "break even" on OMWM costs after 10 years with 10% inflation per year if an area would have been larvicided five times per year for each of the 10 years, if not subjected to OMWM. If the ditch life can be assumed to be 15 years, it is economically feasible to undertake an OMWM project even if the area is larvicided less than three times per year over the 15 year period. Even if inflation is 8% per year for the next 15 years, it is still less expensive to undertake OMWM than to larvicide three times per year.

Despite our relatively low cost per acre for aerial larviciding, it is economically desirable to initiate an OMWM project if the area is a moderate to heavy mosquito breeding area. Therefore, it is clear that surveillance data must dictate the areas in which OMWM should be initiated. Of course, if higher aerial larviciding costs are encountered, the long range economy of OMWM will improve even more. The estimated cost of utilizing BTI at 1/2 to 1 gallon finished spray per acre at 1982 prices is ± $7.00/Acre. At this cost OMWM becomes extremely attractive as a long range solution to salt marsh mosquito problems.

References Cited

Grant, E.L. and W.G. Ireson. 1970. Principles of Engineering Economy. Ronald Press Company, New York, New York. 640 pp.

Lesser, C.R. and Saveikis, D. (1979). A study of the impacts of a mosquito control integrated pest management program on selected parameters of the ecology of Chesapeake Bay high marsh communities in Maryland. Grant Report X 003147-01, Maryland Department of Agriculture - unpublished.

TABLE I

IMPACT OF INTEGRATED PEST MANAGEMENT
ON MARYLAND MOSQUITO CONTROL ACTIVITIES

	TEMPORARY CONTROL				PERMANENT CONTROL
			LARVAL CONTROL		
YEAR	PROGRAM GROWTH # AREAS	ADULT CONTROL MALATHION 91%	Abate 1% SG	Abate 4E	OMWM
1977	1566	21,344 gallons	7,060 lbs.	8.0 gal.	1353 Acres
1978	1686	22,115 gallons	13,505 lbs.	603 gal.	1579 Acres
1979	1800	20,568 gallons	24,090 lbs.	629 gal.	1328 Acres
1980	1801	14,723 gallons	22,240 lbs.	735 gal.	2643 Acres
1981	1829	15,822 gallons	16,855 lbs.	1,658 gal.	1856 Acres

The impact of applying pest management concepts to the Maryland Mosquito Control Program from 1977 through 1981. Better timing, increased larviciding and spraying based on need, resulted in a decline in Malathion use while areas sprayed increased 16.8% from 1566 to 1829. Expanding on water management work on the high salt marshes has resulted in a significant reduction in mosquito annoyance and disease potential.

TABLE II

Cost of Aerial Larviciding Per Acre and Cumulative Acres Treated Per Year from 1978 through 1980

Year	Direct Cost Per Acre Per Treatment	Cumulative Acres Treated
1978	$1.51	49,906
1979	$1.55	49,992
1980	$1.75	58,659
AVERAGES	$1.60	52,852

TABLE III

Cost of Open Marsh Water Management Per Acre and Total Acres Treated Per Year from 1978 through 1980

Year	Direct Cost Per Acre	Acres Treated
1978	$124.93	1,579
1979	$158.60	1,328
1980	$ 94.47	2,630
AVERAGES	$126.00	1,846

TABLE IV

Compound Amount Factors for Uniform Series at Fixed Interest Rates Versus Time (See Grant and Ireson 1970)

Years	Percent				
	8	10	12	15	20
5	5.87	6.10	6.35	6.74	7.44
10	14.49	15.94	17.55	20.30	25.96
15	27.15	31.77	37.28	47.58	72.03
20	45.76	57.28	72.05	102.44	186.69
25	73.11	98.35	133.33	212.79	471.98
30	113.28	241.33	241.33	434.74	1181.88

TABLE V

Comparison of Aerial Larviciding Costs Per Acre Per Year and Total Costs Over a 10 and 15 Year Period

No. of Larvicide Applications Per Year	Cost Per Acre Per Treatment	Cost Per Acre Per Year	Total Cost Per Acre Over A 10 Year Period @ 10% Inflation Per Year	Total Cost Per Acre Over a 15 Year Period @ 10% Infla. Per Year
1	$1.60	$1.60	$ 25.50	$ 50.83
2	$1.60	$3.20	$ 51.01	$101,66
3	$1.60	$4.80	$ 76.51	$152.50
4	$1.60	$6.40	$102.02	$203.33
5	$1.60	$8.00	$127.52	$254.16

APPENDIX A

Heavy Equipment Rental Fees
Period 1978 Through 1980

Type of Equipment	1978	1979	1980
Imp	$60/wk.	$60/wk.	$80/wk.
Tractor/Backhoe	$10/hr.	$10/hr.	$12/hr.
Amphibious Clamshell Ditcher	$18/hr.	$20/hr.	$24/hr.
Amphibious Rotary Ditcher	$22/hr.	$26/hr.	$30/hr.